

**THE NEURAL CONTROL OF MASCULINE REPRODUCTIVE AND
SOCIAL BEHAVIOURS IN THE COMMON MARMOSET
(CALLITHRIX JACCHUS)**

S.A.C. LLOYD

Thesis submitted for the degree
of Doctor of Philosophy to the
University of Edinburgh.

May 1989.



CONTENTS

DECLARATION	vii
ACKNOWLEDGEMENTS	viii
ABSTRACT	x
CHAPTER 1 - General introduction	1
1.1 Introduction	2
1.2 Early work and principles	2
1.3 Neuroanatomy of the hypothalamus and related structures	3
1.4 Control systems within the hypothalamus	11
1.4.1 Feeding behaviour	12
1.4.2 Temperature regulation	14
1.4.3 Cardiovascular regulation	14
1.4.4 Neuroendocrinology	15
1.4.4.1 Posterior pituitary	16
1.4.4.2 Anterior pituitary and intermediate lobe	18
1.4.5 Reproductive and social behaviours	19
CHAPTER 2 - The neural control of masculine reproductive and social behaviours	21
2.1 Masculine sexual behaviour	22
2.1.1 Early work and principles	22
2.1.2 The POA - a sexually dimorphic structure	24
2.1.3 The MPOA-AH	26
2.1.3.1 Evidence for its importance in the control of masculine sexual behaviour	26
2.1.3.2 Does the MPOA-AH control sexual arousal or copulatory activity?	31

2.1.3.3	Importance of the central action of steroid hormones within the MPOA- AH	33
2.1.3.4	Evidence for a role for the MPOA-AH in the hypothalamic-pituitary axis	38
2.1.3.5	Neurotransmitter systems within the MPOA-AH	39
2.1.4	The connections of the MPOA-AH	49
2.1.4.1	The medial forebrain bundle (MFB)	50
2.1.4.2	The stria terminalis (ST)	52
2.1.5	Other areas of importance	53
2.1.5.1	The olfactory system	53
2.1.5.2	The amygdalae	56
2.1.5.3	The neocortex	58
2.1.5.4	The dorsomedial (DMH) and posterior (PH) hypothalamus	60
2.1.6	Penile erectile mechanisms	61
2.2	Parental behaviour	66
2.2.1	Maternal vs. paternal behaviour	66
2.2.2	The MPOA	68
2.2.2.1	Evidence for its importance in the control of parental behaviour	68
2.2.2.2	The importance of the central action of hormones within the MPOA	69
2.2.3	The connections of the MPOA	70
2.2.4	Other neural areas	72
2.2.4.1	The olfactory system	72
2.2.4.2	The amygdalae	75
2.2.4.3	The neocortex	76
2.2.4.4	Other limbic structures	77
2.2.5	Neurotransmitters implicated in the control of parental behaviour	78
CHAPTER 3	- Materials and methods	80
3.1	Introduction	81
3.2	The common marmoset (<i>Callithrix jacchus</i>)	81

4.2 Materials and methods	113
4.2.1 Animals	113
4.2.2 Surgery	113
4.2.3 Testing regime	113
4.2.4 Behaviours scored	114
4.2.5 Blood sampling and assay	115
4.2.6 Histology	115
4.3 Results	116
4.3.1 Lesion placement	116
4.3.2 Effects on sexual behaviour	128
4.3.2.1 Sexual arousal	128
4.3.2.2 Copulatory behaviour	132
4.3.3 Effects on social behaviour	138
4.3.3.1 Grooming behaviour	138
4.3.3.2 Scent marking and genital presents	140
4.3.4 Effects of oestrogen administration to the females	141
4.3.5 Effects on agonistic encounters with a strange male	143
4.3.6 Effects on plasma testosterone levels and androgen-dependent morphology	145
4.3.7 Effects on general health	148
4.5 Discussion	149

CHAPTER 5 - Experiment 2: Oxytocin, β -endorphin and masculine sexual and social behaviour in a pair test situation

157

5.1 Introduction	158
5.2 Materials and Methods	159
5.2.1 Animals	159
5.2.2 Protocol for plasma oxytocin measurement	159
5.2.2.1 Male testing regime and blood sampling	159
5.2.2.2 Female testing regime and blood sampling	159
5.2.3 Surgery (Cannulations)	160
5.2.4 Intracerebral infusion technique	160
5.2.5 Behaviours scored	161
5.2.6 Histology	161

5.3 Results	161
5.3.1 Plasma oxytocin following ejaculation and during vaginocervical stimulation	161
5.3.2 Histology	162
5.3.3 Effects of central administration of oxytocin	168
5.3.4 Effects of central administration of β -endorphin	174
5.3.5 Effects of repeated infusions	175
5.4 Discussion	178

CHAPTER 6 - Experiment 3: POA-AH lesions and masculine sexual and paternal behaviour in a permanent group situation

183

6.1 Introduction	184
6.2 Materials and methods	184
6.2.1 Animals	184
6.2.2 Surgery	185
6.2.3 Video recording	185
6.2.4 Testing regime	185
6.2.5 Behaviours scored	186
6.2.6 Histology	186
6.3 Results	187
6.3.1 Lesion placement	187
6.3.2 Effects on carrying time	192
6.3.3 Effects on transfers	195
6.3.4 Effects on grooming behaviour	197
6.3.5 Effects on developmental markers	200
6.3.6 Effects on sexual behaviour	201
6.3.7 Effects on general activity levels	206
6.3.8 Effects on androgen-dependent morphology	206
6.3.9 Effects on general health	208
6.4 Discussion	209

CHAPTER 7 - Experiment 4: Olfactory bulb sections and masculine sexual and paternal behaviour in a permanent group situation

217

7.1 Introduction	218
7.2 Materials and methods	218
7.2.1 Animal selection	218
7.2.2 Surgery	219
7.2.3 Video recording	219
7.2.4 Testing regime	220
7.2.5 Behaviours scored	220
7.3 Results	221
7.3.1 Olfactory discrimination tests	221
7.3.2 Effects on carrying time	222
7.3.3 Effects on transfers	223
7.3.4 Effects on grooming	224
7.3.5 Effects on sexual behaviour	226
7.3.6 Effects on general health	229
7.4 Discussion	230
 CHAPTER 8 - General Discussion	 236
 REFERENCES	 247
 PUBLICATIONS	 279

DECLARATION

Except where acknowledgement is made by reference, the experiments described in this thesis were the unaided work of the author.

No part of this work has already been accepted for any other degree, nor is any part of it being concurrently submitted in candidature for another degree.

S.A.C. Lloyd, 1989

ACKNOWLEDGEMENTS

I am indebted to a large number of people, all of whom have helped me throughout my three years in Edinburgh in many ways.

Firstly, none of this work would have been possible without the financial support of the Medical Research Council and without the help and encouragement of the staff of the Unit, particularly the Director, Professor Dennis Lincoln.

My particular thanks go to my supervisor Alan Dixson who has given constant practical help, advice, encouragement and friendship whenever required, and to my other supervisor, John Deag, who has helped during my third year in particular.

The staff of the Primate Facility on Bush Estate, Keith, Fiona, Kathryn and Derek have done many things to help with the general maintenance of the marmoset colony and, more specifically, assisting with surgery, blood sampling, changing video tapes and a myriad of other small tasks. I am particularly grateful to Kathryn for all the work that she did with the cannulated males during the infusion study.

The hormonal data that I have quoted in this thesis would not have been possible without the assistance of a number of people: Mairwen Crosby who taught me the testosterone RIA, Steve Lunn who assayed some samples for LH, Jean Wickings who carried out some progesterone assays for me and Keith Kendrick who also assayed some samples for oxytocin at AFRC Babraham in Cambridge.

Steve Lunn also spent many hours explaining the video recording system to me and I benefited greatly from the experience that he had gained whilst carrying out his own behavioural study, for which I am extremely grateful.

Many people have also assisted me with histology and photography, in

particular, Kay Grant (Physiology Dept. Edinburgh), Tom McFetters and Ted Pinner, Mike Martin (MRC Brain Metabolism Unit, Edinburgh) and Ken Donachie.

Many other people have given advice and practical help whether asked or not, and I would like to thank Kevin O'Byrne specifically for his help and friendship.

Finally, my greatest debt of thanks is to my husband Chris, without whom I would never have been writing this thesis. He has given me constant encouragement, practical help - particularly with the presentation of this thesis - and has also written a number of computer programmes to aid analysis of data. Above all, he has provided the constant nagging that all PhD students require at times throughout the course of the three years!

ABSTRACT

The medial preoptic area (MPOA) and anterior hypothalamus (AH) have been implicated in the neural control of masculine sexual behaviour in a wide range of vertebrate species. Some previous research has presented evidence for a role for the medial preoptic area-anterior hypothalamic continuum (MPOA-AH) in the control of sexual arousal and copulatory behaviour, whilst other studies have reported that only aspects of copulatory behaviour are influenced by this neural area. Furthermore, the MPOA has also been implicated in the control of maternal behaviour in a number of mammalian species.

The present series of experiments was undertaken to elucidate the precise role of the MPOA-AH in the control of masculine sexual arousal and copulation, and to determine whether the paternal behaviour exhibited by common marmosets is controlled by the same neural areas that influence the maternal behaviour shown by other mammals. However, surgical techniques were more far-reaching than anticipated, leading to the necessity to discuss the preoptic area (POA) and POA-AH in general rather than the MPOA and MPOA-AH as described in much of the literature. Very little work has been carried out on the neural control of masculine sexual behaviour in primates and, in addition, the present study is the first in which the hypothalamic control of parental behaviour has been investigated in any primate species. Aspects of the males' social and agonistic behaviour were also investigated and, in a preliminary study, the role of olfaction in masculine sexual and parental behaviour was considered.

The sexual and social behaviours of 14 adult male marmosets was measured during pair tests with ovariectomised females before and after placement of thermal (n=10) or sham lesions (n=4) in the hypothalamus.

During post-operative tests, an immediate and pronounced decrease in precopulatory behaviour (anticipatory erections, anogenital investigation of the female and precopulatory tongue flicking) and copulatory patterns (mounts, intromissions and ejaculations) occurred in lesioned subjects. Maximal effects occurred when males were lesioned within the preoptic area-anterior hypothalamic continuum (POA-AH). These effects were not due to decreased levels of circulating testosterone, as evidenced by plasma levels and by androgen-dependent morphology. Treating the females with oestradiol 17 β activated proceptivity but the males showed no associated increases in sexual activity. Preliminary data also indicates that these lesions caused a decrease in inter-male aggression.

The broader social consequences of POA-AH lesions were studied using 6 males in a permanent group situation. Males were lesioned (n=3) or sham lesioned (n=3) on the 11th postpartum day of their female partners. Severe deficits in paternal carrying of the infants and masculine sexual behaviour occurred in all POA-AH lesioned males, however, some recovery of paternal behaviour was seen in 2 cases. These deficits were not due to non-specific behavioural effects, and sectioning the olfactory bulbs in 2 males reduced grooming behaviour and disrupted the patterning of postpartum sexual behaviour, but had no influence upon infant carrying behaviour.

Significant increases in grooming activity were noted following POA-AH lesions in males tested under both conditions, possibly indicating the presence of displacement activities.

In an attempt to elucidate the role of 2 neuroactive peptides in the control of masculine sexual behaviour, stainless steel cannulae were implanted bilaterally into the hypothalamus in 9 male marmosets. Microinfusions of oxytocin or β -endorphin proved to be without effect upon sexual behaviour during pair tests, except for a trend towards stimulation of erection (oxytocin)

and increased mount latencies (β -endorphin) in some subjects. These preliminary studies show that the marmoset is a suitable primate for behavioural studies involving chronic cannulation and microinfusions into the hypothalamus.

The conclusions drawn from the study are that the POA-AH is crucially important in the neural control of sexual, aggressive and parental behaviours in the male common marmoset, and that its role may be in linking the correct behavioural response with the sensory inputs that indicate the sex and social status of the stimulus animal.

CHAPTER 1

GENERAL INTRODUCTION

1.1 Introduction

This chapter briefly describes some of the early work carried out on the neural mechanisms of control of motivational behaviours which highlights the hypothalamus as an important control centre. Therefore, it is with an outline of the neuroanatomy of the hypothalamus and a description of its functions that this thesis begins. The evidence to implicate intra- and extra-hypothalamic regions in the control of masculine reproductive and social behaviours is outlined in Chapter 2.

1.2 Early work and principles

The hypothalamus constitutes less than 1% of the adult human brain, yet its functions are myriad and of paramount importance to the physical and emotional well-being of the individual.

There is a long history of research into role of the hypothalamus as a control centre, which has its roots in a statement made by Claude Bernard in the last century describing the difference between the internal (*milieu interieur*) and external (*milieu exterieur*) environment of an organism:

The living organism does not really exist in the *milieu exterieur* - the atmosphere it breathes, salt or fresh water if that is its element - but in the liquid *milieu interieur* formed by the circulatory organic liquid which surrounds and bathes all the tissue elements; this is the lymph and the plasma The *milieu interieur* surrounding the organs and tissue and their element never varies. . . . Here we have an organism which has enclosed itself in a kind of hot house. The peripheral changes of external conditions cannot reach it; it is not subject to them, but is free and independent. . . . All the vital mechanisms, however varied they may be, have only one object, that of preserving constant the conditions of life in the internal environment.

Claude Bernard, 1878.

Many of the 'vital mechanisms' referred to are situated within the hypothalamus and, together, they constitute one of its major functions - the maintenance of homeostasis.

The role of the hypothalamus in the control of motivational behaviours became clear slightly later, in the 1930s, and this was preceded by the definition of the 'limbic system' - a series of structures within the CNS which is considered to be the 'anatomical substratum for emotions' (Papez, 1937). In this original definition, the limbic system was described as consisting of the cingulate gyrus, hippocampal formation, fornix, mammillary bodies and anterior thalamic nuclei. This system was later expanded to encompass parts of the hypothalamus, the septum, nucleus accumbens, amygdalae and orbitofrontal cortex. Papez's proposal was influenced by earlier work of Hess and Ranson (Kandel & Schwartz, 1985); and, shortly after his paper was published, work by Kluver and Bucy showed that the amygdalae (also part of the limbic system) are also involved in the control of emotions and, therefore, of social behaviour (Kluver & Bucy, 1937). These papers form the basis for the subsequent interest in the role of the limbic system in general, and the hypothalamus in particular, in the control of emotional responses and motivational behaviour.

It is this aspect of neural control that has been investigated in this thesis, with reference to the control of reproductive and social behaviours - which contain elements of emotional and motivational responses to a situation.

1.3 Neuroanatomy of the hypothalamus and related structures

Most of the anatomical studies that are quoted in this section have been carried out using rodents. However, it is thought that the general anatomical

principles are consistent between rodents and primates though results from studies on primates are quoted where possible.

The hypothalamus is thought to act as a link between the special senses and higher levels of consciousness, and the motor systems and reflexive activity of the lower brain stem (Kandel & Schwartz, 1985). It is this functional position within the CNS that makes the hypothalamus so important in the control of motivational behaviours and its anatomical position reflects this.

The hypothalamus is part of the diencephalon, located under the thalamus in the mid brain. As previously mentioned, it is considered to be part of the limbic system and all the component parts of this structure shown in Fig. 1.1 have been implicated in the control of emotional responses.

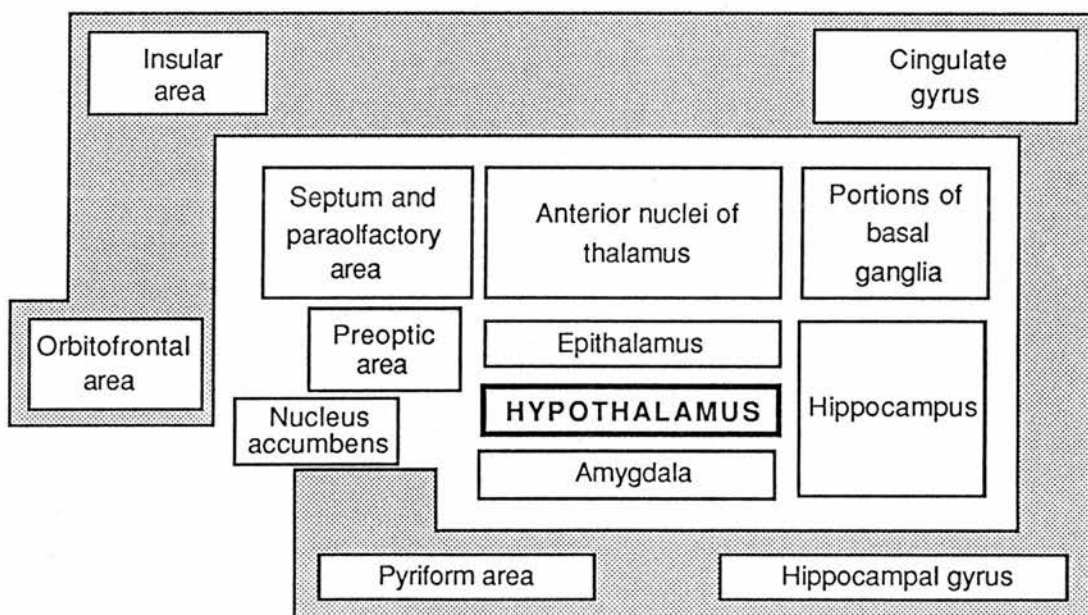


Fig. 1.1 The relationship of the hypothalamus to the other major components of the limbic system.

Many of the major connections of the hypothalamus are with other structures

within the limbic system - see Fig. 1.2. Projections to and from areas caudal to the hypothalamus are carried in the medial forebrain bundle, the mamillo-tegmental tract, and the dorsal longitudinal fasciculus. Other rostral structures are interconnected with the hypothalamus via the mamillothalamic tract, fornix and stria terminalis.

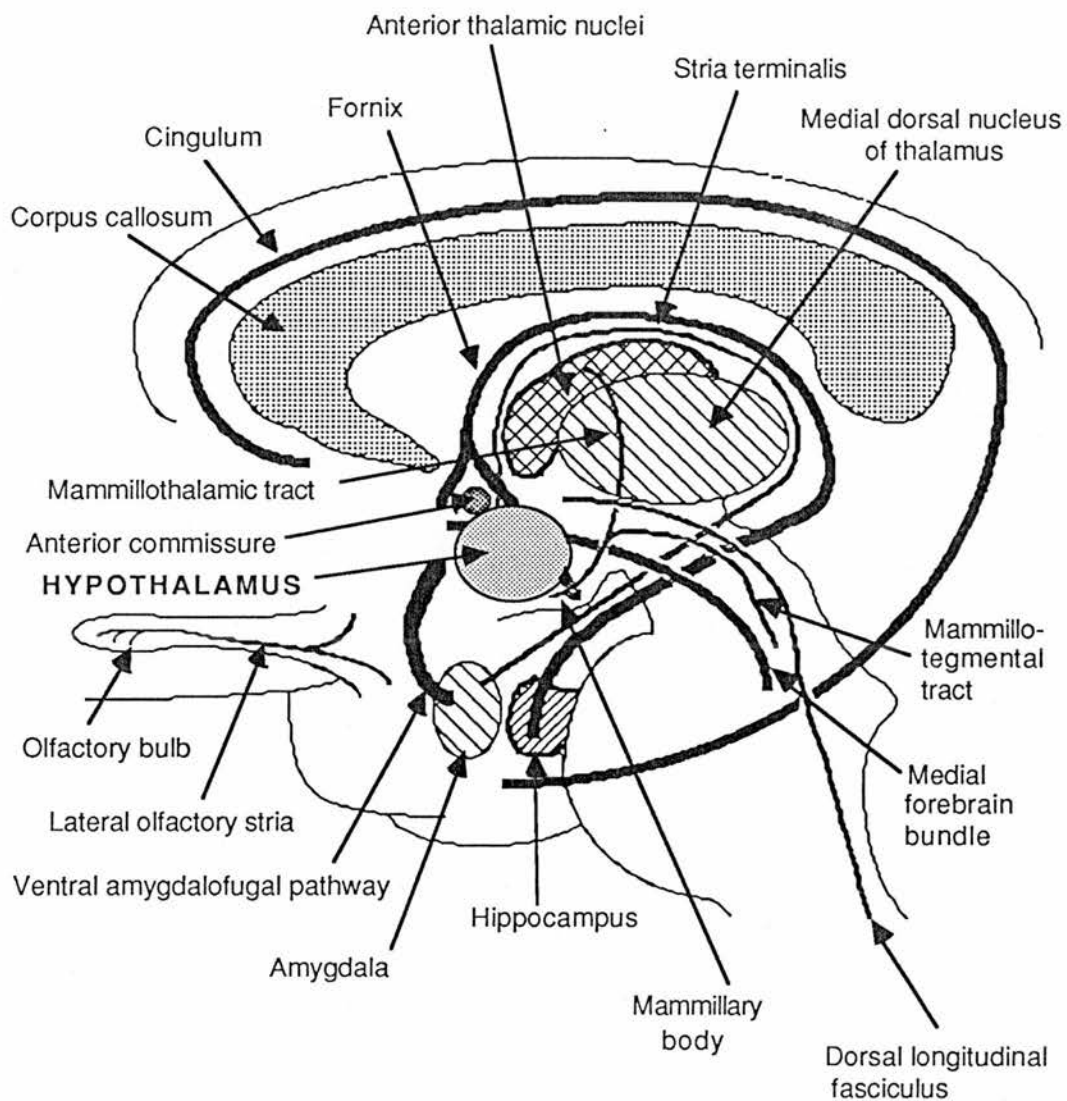


Fig. 1.2 The hypothalamus and its major connections.

Most of the fibre systems of the hypothalamus are bidirectional. One

exception is the hypothalamohypophyseal tract; this tract contains the axons of the paraventricular and supraoptic neurons, which terminate primarily in the posterior pituitary. The hypothalamus also receives one-way afferent connections directly from the retina that terminate primarily in the suprachiasmatic nucleus.

The central importance of the hypothalamus to the function of the limbic system is paralleled by its central anatomical position - see Fig. 1.2.

The internal anatomy of the hypothalamus reflects its diverse functions. One of the primary functions, to control the pituitary gland, can be inferred by its position dorsal to the pituitary, to which it is attached by the infundibulum - see Fig. 1.3. The posterior extent of the hypothalamus is delimited by the mammillary bodies, the anterior extent by the optic chiasm, preoptic area and lamina terminalis.

The hypothalamus can be grossly divided into periventricular, lateral and medial regions. The periventricular area consists of the parts of the hypothalamus bordering the third ventricle. The lateral region has extensive short-fibre, multisynaptic ascending and descending pathways. Most prominent of these is the medial forebrain bundle which runs through the lateral hypothalamus to terminate in various regions of the forebrain. Many aminergic neurons originating in the brain stem course to neocortical regions through fibres in the medial forebrain bundle and its rostral continuation in the cingulum bundle, and these aminergic pathways within the marmoset brain have been mapped in detail (Schofield & Dixon, 1982). The medial region is separated from the lateral region by the descending columns of the fornix and the medial region contains most of the well-delineated nuclear groups of the hypothalamus. The basal portion of this medial region contains many of the hypothalamic neurons that secrete the peptide-releasing factors considered in Section 1.4.

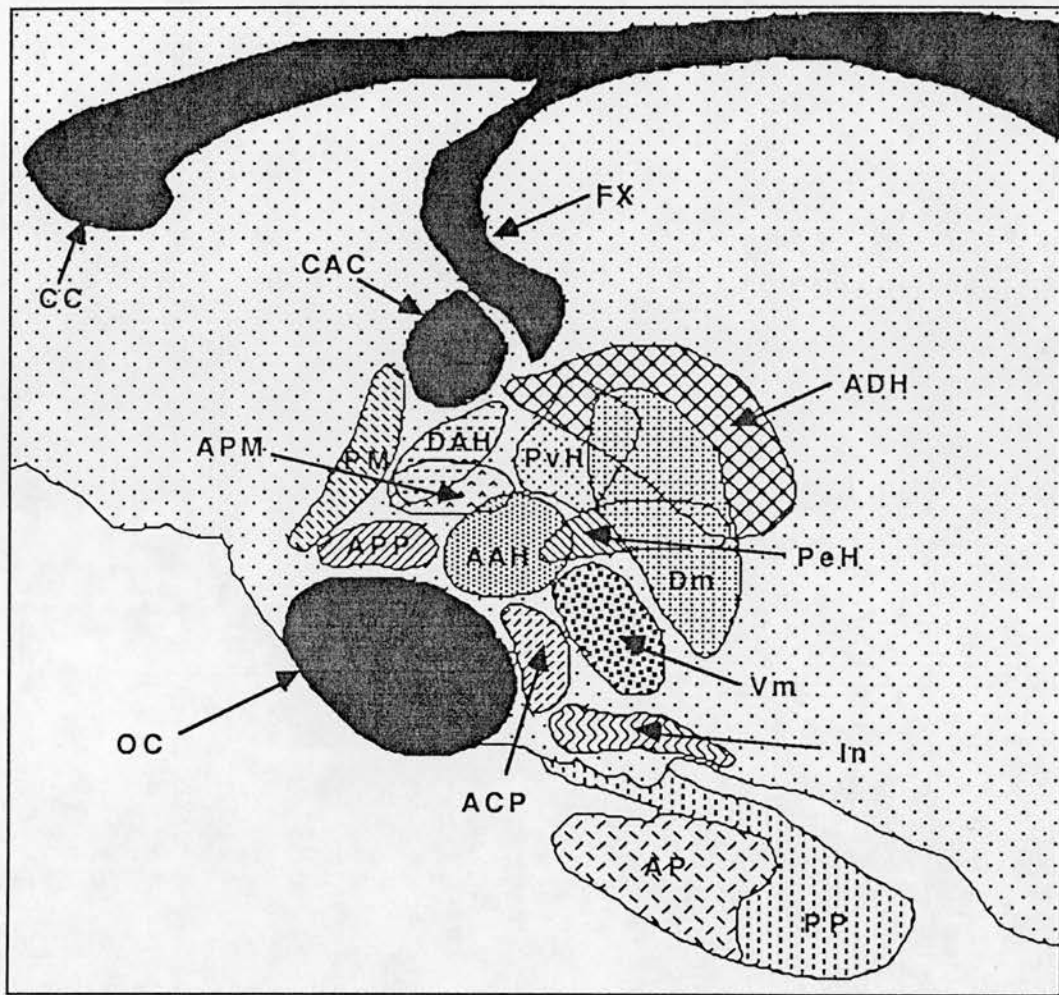


Fig. 1.3 The internal structure of the anterior and medial portions of the marmoset hypothalamus showing the major nuclei - lateral nuclei are not shown.

Key:

CC	Corpus callosum	FX	Fornix
CAC	Anterior commissure	ADH	Dorsal hypothalamic area
DAH	Dorsalis anterior nucleus	* PM	Nucleus preopticus medianus
PvH	Paraventricular nucleus	* APM	Medial preoptic area
* APP	Preoptic periventricularis nucleus	AAH	Anterior hypothalamic area
PeH	Periventricular nucleus	OC	Optic chiasm
ACP	Area commissurae postopticae	Vm	Ventromedial nucleus
Dm	Dorsomedial nucleus	In	Arcuate nucleus
AP	Anterior pituitary	PP	Posterior pituitary

* these structures, together with the lateral preoptic area (LPOA), form the 'POA'

The preoptic area (POA) and anterior hypothalamus (AH) occupy the most rostral portion of the hypothalamus. They are closely interconnected and many of their afferent and efferent connections are common to both structures, originating or terminating in the continuum between them.

The POA is further subdivided into a number of areas; the medial preoptic area (MPOA), normally considered to be limited to the APM (see Fig. 1.3), and the lateral preoptic area (LPOA). Another important component of the POA is the PM - the nucleus preopticus medianus - which is situated medially above the APM.

The MPOA has been found to be sexually dimorphic in a number of mammalian species (see Chapter 2 for details) and occupies a central position within the POA. This nucleus contains a complex neurotransmitter complement (Simerly et al., 1986; Micevych et al., 1987) and has afferent connections with other areas of the hypothalamus i.e. the mammillary bodies and arcuate nucleus, and more distant afferent projections to the bed nucleus of the stria terminalis, amygdalae, central grey and the mesencephalic and subthalamic locomotor regions - many of these projections being carried in the medial forebrain bundle (Conrad & Pfaff, 1975; Swanson et al., 1987).

In return, the MPOA receives noradrenergic and dopaminergic inputs from the brain stem, as well as other inputs from the lateral septum, amygdalae, ventral subiculum, periaqueductal grey, raphe nuclei, dorsolateral tegmental area, locus coeruleus and lateral reticular nucleus (Day et al., 1980; Berk & Finkelstein, 1981). There are also serotonergic inputs from the raphe nuclei which terminate primarily in the lateral part of the MPOA (Simerly & Swanson, 1986). The MPOA is also closely connected with the stria terminalis which links it with the amygdalae and the olfactory system (Heimer & Nauta, 1969; De Olmos & Ingram, 1974). The LPOA receives

afferent inputs from the prefrontal cortex, nucleus accumbens, diagonal band and olfactory structures, septum, brain stem structures and other hypothalamic nuclei (Wayner et al., 1983).

There is also evidence that the POA receives inputs from caudal and rostral brain stem reticular structures (Mallick et al., 1984).

The afferent and efferent connections of the AH are similar to those of the POA. Intra-hypothalamic efferent projections include those to the ventromedial hypothalamus (VmH), arcuate nucleus and mammillary bodies, whilst extra-hypothalamic efferents connect the AH with the septum, bed nucleus of the stria terminalis, amygdalae, ventral tegmental area and fornix (Conrad & Pfaff, 1975).

In return, the AH receives inputs from the lateral septum, amygdalae, ventral subiculum, periaqueductal grey, raphe nuclei, dorsolateral tegmental area, locus coeruleus and lateral reticular nucleus (Berk & Finkelstein, 1981). The AH, like the POA, has major connections with the stria terminalis. A dorsal subventricular portion of the stria terminalis divides into retrocommissural and supracommissural contingents which both have connections with the medial preoptic area - anterior hypothalamic junction area (MPOA-AH). In addition, a ventral juxtacapsular portion of the stria terminalis projects to the MPOA-AH (De Olmos & Ingram, 1974).

The paraventricular nucleus (PvH) lies caudal to the AH and its efferent connections are markedly different. The PvH synthesizes the neurohypophysial hormones arginine vasopressin (AVP) and oxytocin. Therefore, there are major connections with the posterior pituitary gland via the median eminence. However, projections of both AVP- and oxytocin-containing neurons are found leading to other extra-hypothalamic sites. AVP- and oxytocin-containing fibres originating in the PvH have been traced to the dorsal and ventral hippocampus, amygdalae, substantia nigra

and to the substantia gelatinosa of the spinal cord (Buijs, 1978); in fact, the PvH is the only site of origin of oxytocin-containing neurons projecting to the brain stem (Lang et al., 1983).

Afferent projections to the PvH have been found to originate in the lateral septum, amygdalae, ventral subiculum, periaqueductal grey, raphe nuclei, dorsolateral tegmental area, locus coeruleus and lateral reticular nucleus (Berk & Finkelstein, 1981).

The VmH is a large nucleus situated in the medial region of the hypothalamus above the arcuate nucleus and median eminence. Afferent projections include fibres from the lateral septum, amygdalae, ventral subiculum, periaqueductal grey, raphe nuclei, dorsolateral tegmental area, locus coeruleus and lateral reticular nucleus (Berk & Finkelstein, 1981). The VmH also receives major inputs from the supracommissural and ventral juxtacapsular portions of the stria terminalis (Heimer & Nauta, 1969; De Olmos & Ingram, 1974). The efferent pathways from the VmH include those to the midbrain central grey which are of particular importance in the control of the lordotic reflex in female rats (Pfaff, 1980).

The dorsomedial nucleus (DmH), posterior hypothalamus (PH) and lateral hypothalamus (LH) share many of the afferent projections common to other nuclei within the hypothalamus; namely, the lateral septum, amygdalae (except the PH), periaqueductal grey, raphe nuclei, locus coeruleus, dorsolateral tegmental area and lateral reticular nucleus. In addition, all three areas receive afferent inputs from the lateral habenular nucleus and the PH also receives inputs from the diagonal band of Broca (Berk & Finkelstein, 1981).

1.4 Control systems within the hypothalamus

Many of the control systems within the hypothalamus have now been attributed to specific internal areas and nuclei, as can be seen from Fig. 1.4, which details the control of non-sexual functions.

AREA / NUCLEUS	FUNCTION
MPOA	Heat dissipation, cardiovascular control, bladder contraction, neuroendocrinology
LH	Feeding & thirst centre, aggression / rage
AH	Temperature regulation, sweating, panting
PvH	Neuroendocrinology, water conservation
SON	Neuroendocrinology, water conservation
Arcuate nucleus	Neuroendocrinology
VMH	Satiety centre
DMH	GI stimulation
PH	Heat conservation, pupillary dilation
Mammillary bodies	Feeding behaviour.

Fig. 1.4 The non-sexual functions of the major areas / nuclei within the hypothalamus.

Key: MPOA Medial preoptic area LH Lateral hypothalamus
AH Anterior hypothalamus PvH Paraventricular nucleus
SON Supraoptic nucleus VmH Ventromedial nucleus
DmH Dorsomedial nucleus PH Posterior hypothalamus

However, it is important to remember that the brain is not defined clearly in terms of discrete centres that control specific functions; rather, individual functions are performed by neural circuits distributed among several

structures in the brain. It is important to consider all functions of the hypothalamus when interpreting the results of hypothalamic manipulations as many of the gross techniques may result in complex effects involving more than one control mechanism. Much of the early work on the localisation of hypothalamic function was carried out by Hess (review - 1954) using an intracranial stimulation technique, and this series of studies has formed the basis of subsequent work. This section gives an outline of the non-sexual control mechanisms found within the hypothalamus and is not intended to be an exhaustive review of the literature.

1.4.1 Feeding behaviour

Food intake is thought to be under the control of two centres within the hypothalamus. In 1942, it was found that destruction in the region of the ventromedial hypothalamic nuclei and surrounding tissue produces hyperphagia, which results in severe obesity (Hetherington & Ranson, 1942). In contrast, lesions of the lateral hypothalamus produce the opposite effect - a severe aphagia from which the animal dies unless force-fed and hydrated (Anand & Brobeck, 1951). Electrical stimulation of the hypothalamus produces the opposite effects - lateral stimulation elicits feeding behaviour and ventromedial stimulation suppresses it. These findings have led to the hypothesis that the lateral hypothalamus contains a 'feeding centre', and the ventromedial hypothalamus a 'satiety centre'.

The results of several experiments have indicated that hypothalamic lesions may alter the set point for regulating body weight. In these experiments the animal's weight is changed by force-feeding or starvation before lesioning. After a relatively small lateral hypothalamic lesion is made, animals eventually resume eating, although ordinarily at a reduced level of intake. However, if the weight of the animals is reduced before the lateral

hypothalamic lesion, the animals eat and gain weight immediately after the lesion, instead of losing weight as the control (non-starved) animals do. This prestarvation apparently brings the animals' weights below the set point determined by the lateral lesion. Analogous but converse results are obtained when animals are force-fed and then given hypothalamic lesions that ordinarily result in over-eating (Keesey et al., 1976).

Feeding behaviour is affected by many hormones including sex steroids, growth hormone, glucagon and insulin. Large lesions of the hypothalamus invariably affect many hormonal control systems. For example, lesions of the medial hypothalamus result in a greatly increased release of insulin when animals are exposed to food and this may, in part, explain the hyperphagia seen after medial hypothalamic lesions (Kandel & Schwartz, 1985).

Lesions of the lateral hypothalamus also damage fibres of passage and it is possible that some of the effects seen post-lesion are due to this. Two fibre pathways are of particular importance here; the dopamine-containing pathway coursing through the lateral hypothalamus from the substantia nigra to the striatum, and the fibres of the trigeminal system. In the case of the former fibres, sectioning outside the hypothalamus causes a hypoarousal state and aphagia similar to that seen following lateral hypothalamic lesions. In the case of the fibres of the trigeminal system, the resultant sensory loss on lesioning can contribute to aphagia. Sectioning of the peripheral trigeminal input can also disturb feeding behaviour.

This control system illustrates clearly the necessity to distinguish between the functions of the cell bodies within an area of the CNS and those of the fibres of passage coursing through the structure, which may also be damaged during lesioning or other chronic manipulations. This important point will be discussed further in subsequent chapters.

1.4.2 Temperature regulation

Electrical stimulation of the anterior hypothalamus, particularly the preoptic area, in unanaesthetized animals results in dilation of blood vessels in the skin and suppression of shivering - responses that result in a drop in body temperature. Electrical stimulation of the posterior hypothalamus produces a set of opposite responses that result in conservation of body temperature (Hayward, 1977).

Although both anterior and posterior hypothalamic areas are involved in temperature regulation, detectors of temperature - both low and high - are located only in the anterior hypothalamus and preoptic area. The hypothalamic receptors are probably neurons whose firing rate is highly dependent on local temperature, which in turn is determined primarily by the temperature of the blood (Hori et al., 1987). The signals from these internal temperature receptors are integrated from messages received from other visceral and subdermal receptors. It would appear that prostaglandins influence this central control of body temperature by the POA, as infusions of PGE into this area produce hyperthermia (Matsumura et al., 1988).

The hypothalamus also controls endocrine responses to temperature challenges. Thus, long-term exposure to cold can enhance an animal's release of thyroxine thereby increasing body temperature by increased tissue metabolism.

1.4.3 Cardiovascular regulation

The hypothalamus is also known as the head ganglion of the autonomic nervous system, and it is in this capacity that it regulates the cardiovascular system.

Stimulation of different areas throughout the hypothalamus can cause every known type of neurogenic effect in the cardiovascular system, including

increased or decreased arterial pressure and increased or decreased heart rate (Hayward, 1977). In general, stimulation of the posterior and lateral hypothalamus results in increases in arterial pressure and heart rate, whilst stimulation in the preoptic area results in the opposite effects (O'Neill & Brody, 1987). These effects are transmitted mainly through the cardiovascular control centres in the reticular substance of the medulla and pons (Guyton, 1981). The effects of the POA on peripheral vasculature and blood pressure have been cited as a possible cause of the lack of sexual activity seen after POA lesions in males, and this point will be discussed in more detail in Chapter 2.

1.4.4 Neuroendocrinology

One of the major functions of the hypothalamus is to control the release of hormones from the posterior and anterior pituitary gland. This control is accomplished in two ways; either by direct release of hormones into the circulation via the posterior pituitary i.e. oxytocin and vasopressin, or by the release of stimulating and inhibiting substances into the local portal plexus in the median eminence, which then drains into the blood vessels of the anterior pituitary. These hypothalamic regulating hormones in turn control the release of hormones into the general circulation.

The two types of neuroendocrine control (direct and indirect) are mediated by two classes of peptidergic neuroendocrine cells. In both classes of neurons, the secretory products or precursor peptides are synthesized in the cell bodies, packaged in neurosecretory vesicles that are transported down the axons to the axon terminals and stored there until they are released when the neuron is stimulated. The magnocellular (large) neurons are located in the paraventricular and supraoptic nuclei and are the ones which release oxytocin and vasopressin into the general circulation via the

posterior pituitary. The parvocellular (small) neurosecretory neurons are situated in several hypothalamic regions; in the mediobasal region - in the arcuate and tuberal nuclei - the periventricular region, and also in the preoptic, paraventricular, and suprachiasmatic nuclei. The parvocellular neurons release their secretions into the portal vessels to stimulate or inhibit secretions from the anterior pituitary. The capillaries of the posterior pituitary and median eminence are highly fenestrated to facilitate entry of the neurosecretory products into the blood vessels.

An important point to note here is that oxytocin and vasopressin neurons do not only project to the posterior pituitary - there are other projections which interact with neurons not necessarily involved in the release of hormones (Buijs, 1978; Sofroniew, 1980; Lang et al., 1983).

The regulatory nature of the hypothalamus is influenced by feedback of the secretory products or the substances which they, in turn, control. This is particularly true in the case of the gonadotrophins and sex steroids and is evidenced by the presence of receptors within the hypothalamus for both classes of hormone (Pfaff, 1968, Sar & Stumpf, 1973; Silverman, 1984). However, these hormones have central effects other than a purely self-regulatory mechanism, and details of the specific neuroendocrinological systems that impinge upon the control of masculine reproductive and social behaviours will be discussed in more detail in Chapter 2. The following two sections give an outline of the hormones released from the two lobes of the pituitary, the hypothalamic substances that control this release, and the peripheral functions that these hormones perform.

1.4.4.1 Posterior pituitary

The posterior pituitary secretes two hormones, vasopressin and oxytocin. The former hormone is involved in fluid balance and the latter in uterine

contractions and milk ejection.

The hypothalamus controls fluid balance in two ways; by creating the sensation of thirst and controlling the excretion of urine. The former function is controlled by a 'thirst centre' in the lateral hypothalamus in the same way that feeding behaviour is controlled by this area, and the latter by the paraventricular and supraoptic nuclei.

The paraventricular and supraoptic nuclei contain arginine vasopressin-releasing neurons (Hayward, 1977). Vasopressin alters the membrane permeability of the collecting ducts and convoluted tubules of the kidneys so that their membranes are more permeable to water. As a result, the recovery of water after filtration is facilitated, urinary volume decreased, and body fluid conserved. The neurons that release vasopressin are spontaneously active and provide a basal concentration of the hormone in the blood; this concentration is decreased or increased dependent on the physiological demand. Vasopressin-releasing neurons fire more rapidly when the animal is deprived of water, and less rapidly when the animal is fully hydrated. Vasopressin-releasing cells also respond directly to the osmotic potential of the surrounding fluid and to the local concentration of Na^+ ions. The release of vasopressin is also controlled by neural inputs from blood volume receptors in the peripheral circulation; decreased blood volume enhances vasopressin release and *vice versa*. Afferent input also probably comes from temperature receptors in the skin - cold inhibits the release of vasopressin and warmth enhances it (Kandel & Schwartz, 1985).

The vasopressin-releasing neurons of the paraventricular and supraoptic nuclei project down the infundibulum into the posterior pituitary and it is here that the arginine vasopressin is actually released into the circulation.

Oxytocin is released from the posterior pituitary in a similar way to vasopressin, but it controls a different mechanism. Oxytocin induces

contraction of the myoepithelial cells of the mammary gland and also increases the amplitude of uterine smooth muscle contraction if the muscle has been oestrogen-primed (Kandel & Schwartz, 1985).

In 1974, Lincoln and Wakerley succeeded in recording from identified neuroendocrine cells in the urethane-anaesthetized female rat while the rat was presented with a natural stimulus for oxytocin release - suckling of pups. Milk ejection was simultaneously measured by recording intramammary pressure. They found that a continuous suckling stimulus produced periodic synchronized bursts of action potentials in many of the identified neuroendocrine cells. Approximately 13 seconds after the burst, there was an increase in intramammary pressure, indicating the arrival of a pulse of oxytocin at the mammary glands. Thus, the oxytocin cells participate in a reflex in which the afferent limb is neural and the efferent limb is humoral.

1.4.4.2 Anterior pituitary and intermediate lobe

It is now clear that most hormones of the anterior pituitary and intermediate lobe are controlled by peptide neurohormones synthesized by the parvicellular neurons that release their product into the capillaries of the median eminence. The release of most of the hormones is regulated by both stimulatory and inhibitory substances released from the hypothalamus, and the commonest of these are listed below with an indication of the pituitary hormones that they control (Kandel & Schwartz, 1985):

- i) Thyrotropin-releasing hormone: stimulates release of thyrotropin and prolactin.
- ii) Corticotropin-releasing hormone: stimulates release of adrenocorticotropin and β -lipotropin.
- iii) Luteinizing hormone-releasing hormone (LHRH): stimulates release of luteinizing hormone (LH) and follicle stimulating hormone (FSH).

- iv) Growth hormone-releasing hormone: stimulates release of growth hormone.
- v) Melanocyte-stimulating hormone-releasing factor: stimulates release of MSH and β -endorphin (released from the intermediate lobe).
- vi) Prolactin release-inhibiting hormone: inhibits release of prolactin.
- vii) Dopamine: inhibits release of prolactin (?).
- viii) Somatostatin: inhibits release of growth hormone and thyrotropin.
- ix) Melanocyte-stimulating hormone release-inhibiting factor: inhibits release of MSH.

The hypothalamus has both neural and hormonal outputs and inputs and can therefore participate in four classes of reflex; (i) conventional reflexes involving neural input and neural output, (ii) reflexes in which the input is neural and the output is humoral, (iii) reflexes in which the input is humoral and the output is neural, and (iv) reflexes in which both the input and the output are humoral. The pituitary gland presents a pathway for type (ii) and (iv) reflexes and is very important in this respect. However, it is outside the scope of this review to discuss in detail the physiological role of all the anterior pituitary hormones and their hypothalamic controlling factors. Those hormones that are most relevant to the study of masculine sexual behaviour are FSH and LH and their releasing factor, LHRH, and this aspect of hypothalamic-pituitary control is discussed in greater detail in later Chapters.

1.4.5 Reproductive and social behaviours

It is clear that the hypothalamus is closely involved in the control of many aspects of homeostasis and motivational behaviours and the systems that control the release of gonadotrophins and, therefore of sex steroids, are closely interlinked with these behavioural control mechanisms. It was

therefore decided to begin the investigation of the control of reproductive and social behaviours in a male primate within the hypothalamus, and to use the common marmoset (*Callithrix jacchus*) as an experimental model. The evidence to implicate areas of the hypothalamus in the control of masculine reproductive and social behaviour is outlined in Chapter 2.

CHAPTER 2

THE NEURAL CONTROL OF MASCULINE REPRODUCTIVE AND SOCIAL BEHAVIOURS

2.1 Masculine sexual behaviour

2.1.1 Early work and principles

The study of the role of the hypothalamus in the control of mammalian masculine sexual behaviour began in the 1940s, soon after Papez had proposed his hypothesis that the limbic system was the 'anatomical substratum for emotions' (Papez, 1937).

Early work on the guinea pig indicated that the hypothalamus exerts a stimulatory influence on sexual behaviour i.e. hypothalamic lesions cause a reduction in sexual behaviour post-operatively, with the major effects being caused by lesions situated between the optic chiasm and the pituitary stalk i.e. the anterior hypothalamus (AH) (Brookhart & Dey, 1941; Brookhart et al., 1941). These, and most subsequent studies on a range of mammalian species, involved a series of pre-operative tests followed by surgery and a post-operative test series, with the post-operative tests beginning several days after surgery. Under these conditions, lesions within the hypothalamus generally result in a post-operative decline in masculine sexual activity. An interesting exception to this pattern is found in an early paper by Hillarp et al. (1954) in which lesions in the preoptic area (POA) resulted in masculine hypersexuality in both male and female rats. This seemingly contradictory result may have been due to the fact that, in this study, the rats were tested immediately on recovery from the anaesthetic. It is possible that, at this stage, the full degeneration of neural tissue surrounding the lesion site was not complete, or that the lesion may have caused transitory stimulation of cell bodies in the surrounding area. In most studies, this possible initial stimulation of sexual activity is not seen as most researchers allow their

subjects to fully recover from surgery before beginning the post-operative test series.

These early studies were followed by a number of investigations aimed at defining the precise areas within the hypothalamus that control masculine sexual behaviour, and at separating this control from any possible effects on the hypothalamic-pituitary axis (Soulairac & Soulairac, 1956; Phoenix, 1961; Heimer & Larsson, 1966/67). From these studies it became clear that the AH and POA are particularly important in the control of masculine sexual behaviour in the rat and guinea pig - though lesions in the medial and dorsal regions of the hypothalamus of guinea pigs also resulted in deficiencies in some studies (Phoenix, 1961) - and that these effects are independent of any interference with pituitary or testicular hormones. The latter can also be inferred from the time course of the onset of these deficits; following hypothalamic lesions, sexual behaviour declines immediately, whilst following castration, copulation may continue for many months (Larsson, 1979). Also, treatment with testosterone propionate following hypothalamic lesions does not restore sexual activity in male guinea pigs and rats (Phoenix, 1961; Heimer & Larsson, 1966/67).

The fact that the central action of testosterone is important for the reactivation of copulatory activity following castration however, was first investigated in a study on the intracerebral implantation of crystalline testosterone propionate in castrated rats (Davidson, 1966). In this study, the most effective areas for stimulation of sexual activity by implanted androgens were the AH and POA. Another line of research that was used in early investigations was that of intracranial stimulation, used to elicit erection, seminal emission and sexual behaviour in a variety of mammalian species. Stimulation of the anterior dorsal hypothalamus was found to attenuate sexual activity in male rats (Vaughan & Fisher, 1962); whilst stimulation of a wide variety of intra- and

extra-hypothalamic sites results in erection and / or seminal emission in the squirrel monkey (MacLean & Ploog, 1962; MacLean et al., 1963), the rhesus macaque (Robinson & Mishkin, 1968), and the rat (Herberg, 1963).

From this early work, it is clear that the hypothalamus plays an important role in the control of masculine sexual behaviour, with the AH and POA being particularly important loci. Other intra- and extra-hypothalamic areas have been implicated in the control mechanism and will be discussed in turn. However, it is with a description of the sexual dimorphisms within the POA followed by a description of the role played by the medial preoptic area-anterior hypothalamic continuum (MPOA-AH) in controlling masculine sexual behaviour that this review begins.

2.1.2 The POA - a sexually dimorphic structure

As the POA appears to be so important in the control of masculine sexual behaviour, it is interesting to find that it contains a sexual dimorphism in rats (Raisman & Field, 1971). This has since been characterised as an intensely staining component of the MPOA which is markedly larger in males than in females - the SDN-POA (Gorski et al., 1978). This sexual dimorphism is characterised by a larger volume of the nucleus, and therefore more neurons, in the male than in the female and not by higher cell density. A similar sexual dimorphism has also been described within the POA of the mouse (Torrán-Allerand, 1976), the hamster (Greenhough et al., 1977), the gerbil (Byne & Bleier, 1987) and man (Swaab & Fliers, 1985).

As well as a gross difference in the volume of the SDN-POA between males and females, there are more subtle internal anatomical differences. It has been found that the male SDN-POA contains larger cells and neurons than

the female, though this may be purely due to the difference in total brain volume between the sexes. There are also sex differences in the dendritic structure of the POA - in juvenile monkey brains, neurons of males have more dendritic bifurcations and a higher frequency of spines than those of females (Ayoub et al., 1983). Recently it has been proved that this increase in volume and the number of synapses within the SDN-POA is dependent on oestrogen (Matsumoto et al., 1988) and this is presumably equivalent to the high levels of oestrogen found in the POA of males following the aromatization of testosterone - see section 2.1.2.3.

Neurons destined to become part of the SDN-POA in rats are produced later during embryonic development than those of the non-sexually dimorphic areas of the MPOA. There is also evidence that the neuroblast division which produces the neurons of the SDN-POA may begin earlier, and terminate sooner, in the female than in the male (Jacobsen & Gorski, 1981). It is now clear that this sexual differentiation is closely dependent on gonadal steroids. SDN-POA neurons accumulate oestradiol, testosterone and dihydrotestosterone in adulthood (Jacobsen et al., 1987) and the effects of oestrogen on cholecystokinin immunoreactivity within the SDN-POA varies throughout the oestrous cycle in female rats (Oro et al., 1988). Both these facts indicate the presence of active steroid receptors within the SDN-POA in adulthood. These steroid receptors also play a vital role during embryogenesis to produce the sexual dimorphisms seen in adult rats (Arendash & Gorski, 1987), quails (Panzica et al., 1987) and guinea pigs (Hines et al., 1985; Byne & Bleier, 1987). It is thought that the sexual dimorphisms arise by a neurotrophic action of androgens which prevents cell death within the SDN-POA. Therefore males and androgenised females retain more neurons in this highly steroid-dependent neural area (Arendash & Gorski, 1987).

The SDN-POA is not the only sexually dimorphic structure found in the adult brain, others have been characterized, including those within the amygdalae (Nishizuki & Arai, 1981) and the bed nucleus of the stria terminalis (Hines et al., 1985). However, the SDN-POA is probably the best documented and is of particular interest due to its position within an area that is clearly important in the control of sexually dimorphic behaviours, though its specific function remains unknown at present.

2.1.3 The MPOA-AH

It would appear that the entire continuum from the preoptic area through to the anterior hypothalamus is important in the control of masculine sexual behaviour. In some studies, this whole area (the MPOA-AH) has been studied and in others, there have been more precise manipulations within the discrete nuclei of the POA or the AH. Evidence from both types of study is presented below to give an overall impression of the influence of the MPOA-AH on sexual behaviour though, where necessary, discussion is limited to the function of more discrete locations within the continuum.

2.1.3.1 Evidence for its importance in the control of masculine sexual behaviour

There are three traditional methods of investigation that were originally employed to study the function of the MPOA-AH - lesioning techniques, intracranial stimulation and intracranial recording, though other techniques such as hormone implantation and pharmacological manipulations have been used since. The major findings from each of the original lines of research will be discussed in turn below and results from studies exploiting

other techniques will be discussed in later sections.

i) Lesion studies. Some of the early hypothalamic lesion studies have already been discussed in section 2.1.1. However, since this early work there have been many studies on the role of the MPOA-AH in the control of masculine sexual behaviour using the same technique in a wide range of vertebrate species; the goldfish (Koyama et al., 1984), the frog *Rana pipiens* (Schmidt, 1968), the lizard *Anolis carolinensis* (Wheeler & Crews, 1978), the chick (Meyer, 1974), the rat (Chen & Bliss, 1974; Ginton & Merari, 1977; Klaric & Hendricks, 1986), the gerbil (Yahr et al., 1982), the mouse (Bean et al., 1981), the cat (Hart et al., 1973; Hart, 1980), the dog (Hart, 1974; Hart & Ladewig, 1979), the goat (Hart, 1986) and the rhesus monkey (Slimp et al., 1978). All these studies have shown an abrupt decline in sexual activity post-operatively, indicating that the MPOA-AH contains a phylogenetically ancient facilitatory control mechanism for masculine sexual behaviour.

These former studies have all employed electrolytic or thermal lesions, which result in a discrete hole in the neural tissue destroying all cell bodies and fibres of passage within that area. Therefore, it is possible that some of the effects seen following a lesion are due to the destruction of fibre tracts which course through the MPOA-AH rather than the cell bodies. The use of neurotoxic substances that destroy only cell bodies within an area, and spare axons, has produced results which refute this hypothesis. The neurotoxins used are excitatory glutamate analogues which bind to glutamate receptors found only on cell bodies, and cause cell death by depolarisation of the membranes caused by increased Na^+ conductivity (Schwarcz et al., 1978). There are a number of neurotoxins available including kainic acid, ibotenic acid, N-methyl-D,L-aspartic acid and quisqualic acid and their relative neurotoxic potency varies (Hastings et al., 1985). In most studies, ibotenic acid is the neurotoxin that is employed.

Ibotenic acid has been used in rats and has produced essentially the same results as those from electrolytic or thermal lesion studies - an immediate and dramatic decline in sexual activity (Hansen, 1982a; Hansen et al., 1982a).

In all the studies mentioned so far, no recovery of sexual activity following MPOA-AH lesions has been observed, even after a comparatively long period i.e. 8 months in the rat (Ginton & Merari, 1977). However, when lesions are carried out in prepubertal male rats, some individuals exhibit sexual behaviour in adulthood even after large MPOA-AH lesions, and this is dependent upon the post-lesion rearing conditions. Males that have been raised in heterosexual groups show sexual behaviour in adulthood and those that have been raised in social isolation do not (Twiggs et al., 1978; Leedy et al., 1980). However, sparing of sexual behaviour is not complete, as the animals which show normal levels of male behaviour under standard testing conditions have fewer ejaculations to exhaustion, and show a more rapid decline in sexual behaviour following castration. The authors suggest that the animals reared in social groups gained the vital sensorimotor experiences during play with their peers that are necessary for the expression of sexual behaviour in adulthood. However, evidence from another study would suggest that it is physical contact in general that is important, and not necessarily only play behaviour. In this study, male rats were reared in four conditions; in isolation without handling, in isolation with daily handling, across a perforated divider from a male peer, or together with a male peer (social). Socially reared and handled males, but not isolated and divided males, with MPOA lesions showed evidence of copulatory recovery (Meisel, 1982). These studies suggest that there is a neural plasticity within the MPOA that may negate the effects of large MPOA lesions if followed by the correct rearing conditions, and it was thought that this

plasticity may be related to the animal's hormonal state at the time of lesioning. In an effort to test this hypothesis, Meisel (1983) lesioned juvenile male rats that were treated with testosterone propionate or oil (control) from 10 to 45 days of age. The proportions of lesioned males copulating as adults did not differ for males previously injected with oil or testosterone. In a second experiment, Meisel castrated rats at 15 days of age and lesioned them in adulthood. Following testosterone replacement, these males displayed copulatory impairments regardless of their hormonal state during development. Taken together, the results of the experiments suggest that the plasticity of the MPOA in rats is a function of some aspect of chronological age unrelated to the animal's hormonal condition prior to the time of the lesion. Further experiments suggest that this neural plasticity may be species-specific, as sparing of sexual function was not evident with lesions made in perpubertal dogs (Hart & Ladewig, 1979) and cats (Hart & Leedy, 1985).

ii) Intracranial stimulation. There have also been a number of stimulation studies to follow up the early work using this technique. Electrostimulation of the lateral preoptic area (LPOA) was found to facilitate masculine sexual behaviour in rats in one study (Madlafousek et al., 1970) and not in another (Malsbury, 1971), though in this latter study sexual behaviour was activated by stimulation of the MPOA. This discrepancy may be due to the fact that the former study investigated the medial region of the LPOA and the electrodes may have impinged on the MPOA. The contention that the LPOA does not influence sexual behaviour is further supported by a study by Merari and Ginton (1975).

The specific effects of MPOA stimulation appear to vary between studies and to be partly dependent on the hormonal state of the male. Stimulation of the MPOA in castrated rats results in vigorous mounting behaviour and even a

few intromissions, but no ejaculations (Van Dis & Larsson, 1971). However, intact male rats in this study, and in a later one (Merari & Ginton, 1975), behaved differently. In this case, MPOA stimulation resulted in a great enhancement in the number of ejaculations, and marked decrease in the latency to ejaculation, the post-ejaculatory refractory period, and in the number of intromissions preceding an ejaculation. It is possible that this difference in ejaculatory ability in response to stimulation of the MPOA is due to a lack of gonadal steroids in the castrates. However, a study on intact rhesus monkeys provides evidence against this theory as, in this case, MPOA stimulation resulted in mounts of longer than normal duration but ejaculation did not occur even after multiple stimulus-induced mounts (Perachio et al., 1979). In this latter study, penile erections were also induced by stimulation within the AH in socially isolated or restrained males, though stimulation here was ineffective in provoking sexual behavioural responses when tests were performed on pairing the males with receptive females. This point illustrates the difficulty encountered when trying to translate results from stimulation studies into a physiological response paralleling sexual arousal or copulatory behaviour.

iii) Intracranial recording studies. The final line of evidence for the importance of the MPOA-AH in the control of masculine sexual behaviour comes from intracranial recording studies. Male rhesus monkey MPOA neurons show a high firing rate before the commencement of sexual activity, and decreased activity after acquisition of the female partner in an operant testing situation. The activity further decreases following ejaculation and later gradually recovers with time. In addition, some MPOA neurons transiently increase activity prior to the copulatory act in relation to penile erection (Yoshimatsu, 1983; Oomura et al., 1984).

2.1.3.2 Does the MPOA-AH control sexual arousal or copulatory activity?

An important question to be answered is whether the MPOA-AH is primarily involved in the control of sexual arousal or of copulatory activity. Beach (1956) first utilized the concept of a sexual arousal mechanism to deal with precopulatory behaviour and a copulatory mechanism to deal with responses related to intromission and ejaculation. The meaning of these two phrases in relation to the male common marmoset are described in Chapters 3 and 4 when the specific behaviours expressed by this species are categorised into 'arousal' and 'copulation'. The literature is contradictory on this point and there also appears to be inter-species variation. Indeed, Larsson (1979) views the POA as providing linkages between sexual arousal and copulation in the rat at least.

As previously mentioned, results from most intracranial stimulation studies indicate that MPOA electrodes stimulate penile erection, reduced mount latencies and longer mount durations. However, few intromissions and ejaculations are elicited from electrodes implanted within the MPOA. Some researchers using lesioning techniques have also concluded that the MPOA-AH is primarily controlling sexual arousal and not copulatory behaviour (Chen & Bliss, 1974). This theory is further supported by evidence from intracranial recording studies. As stated earlier, MPOA neurons show a high firing rate before the commencement of sexual activity (Yoshimatsu, 1983). In addition, it has been shown that mate calling - presumably an indicator of sexual arousal - can be induced in freely moving leopard frogs by stimulation of the POA (Wada & Gorbman, 1977) and that the neural 'mate calling circuits' can be triggered in isolated brainstems of the same species by stimulation of the anterior POA (Schmidt, 1984).

However, there is an equally strong line of evidence to support the hypothesis that the MPOA-AH is primarily controlling copulatory activity. This

is mainly supported by evidence from electrolytic and thermal lesion studies in which aspects of sexual arousal are spared. For example, lesioned male goats continue to show a 'flehmen' response to female urine (Hart, 1986), mice still emit precopulatory ultrasonic vocalisations (Bean et al., 1981), hamsters, dogs and cats continue to investigate the anogenital region of females and sometimes attempt to mount (Powers et al., 1987; Hart, 1974; Hart, 1980) and rhesus monkeys continue to exhibit masturbatory behaviour in their home cages (Sлимп et al., 1978). However, this latter behaviour may not be a true measure of sexual arousal, as it occurred whilst the males were in visual and olfactory isolation from females. It may not therefore be equivalent to the erections, masturbation and ejaculations in response to the visual stimulus of a female that has been recorded in chacma baboons (Bielert et al., 1980).

Further evidence for this hypothesis comes from a study in which male rats were trained to perform an operant response to gain access to a female in order to mate with her. POA lesions abolished mounts, intromissions, and ejaculations but did not disrupt instrumental responses, investigation of the female, or abortive mounting attempts suggesting that the males were still sexually aroused but unable to perform ejaculatory mounts (Everitt & Stacey, 1987).

Results from neurotoxic lesion studies in rats also support the second hypothesis. Male rats continue to follow and to sniff at females and sometimes mount, but are unable to perform the stereotyped pelvic thrust pattern which is necessary for intromission (Hansen, 1982a).

It is therefore clear that the precise role of the MPOA-AH in the control of masculine sexual behaviour is still unknown though there is an argument that the effects seen on masculine sexual behaviour following MPOA-AH lesions are not due to an effect on arousal or on copulatory ability but to an

effect on peripheral vasculature. Pfaff (1980) points out that the POA is important in regulating various autonomic functions, and that decreases in sexual activity after lesioning might be due to impairment of the parasympathetic control of erection. Pulmonary oedema due to systemic vasoconstriction can also occur after lesions of the POA, and such effects might influence the males' copulatory behaviour. However, erection is not necessarily impaired by lesions of the POA, and electrical stimulation of this area activates sexual responses in species that lack a penis and in which external fertilisation is the norm (leopard frog: Wada & Gorbman, 1977). In the context of vertebrate evolution, the involvement of the POA in the integration of male sexual behaviour pre-dated the evolution of the mammalian penis.

2.1.3.3 Importance of the central action of steroid hormones within the MPOA- AH

Before considering the importance of the central action of hormones, it is necessary to consider the role that they play in general in the differentiation and maintenance of masculine sexual behaviour.

The basic importance of gonadal steroids (androgens) for the initiation and maintenance of sexual behaviour in male mammals is documented in several ways. Firstly, secretion of gonadal hormones during fetal and early postnatal life is a prerequisite for the differentiation of male genital organs and influences the development of the central nervous mechanisms underlying masculine sexual behaviour (Goy, 1966; Abbott, 1984; Pomerantz et al., 1986; Tobet & Baum, 1987; Jarzab et al., 1987; Tonjes et al., 1987). Secondly, in no mammal are the body functions which are involved in controlling reproductive behaviour fully developed at birth. Their

maturation is dependent on gonadal steroids and can be evoked prepubertally by administration of these hormones (Meyer, 1974; Abbott & Hearn, 1978; Nadler et al., 1987). Thirdly, many mammalian species show a seasonal variation in their reproductive activity and this variation is accompanied by gonadal changes. During the phase of sexual inactivity, the gonads become atrophic in some species assuming a prepubertal appearance (Lincoln, 1981). Fourthly, prepubertal gonadectomy prevents sexual maturation, and castration performed postpubertally results in a decrease and eventual abolishment of sexual activity. These effects may be counteracted by testicular hormones (Zumpe & Michael, 1985; Bean et al., 1986; Schenck & Koos Slob, 1986; De Jonge et al., 1986; Rissman, 1987; Deviche & Moore, 1988).

The main sources of androgens are the testes and the adrenal glands. The principal hormone produced by the testes is testosterone and the adrenals produce several different androgens. These hormones are synthesized and degraded following several pathways. One of the pathways for the metabolism of testosterone involves ring A reduction through a widely occurring enzyme - 5α -reductase leading to dihydrotestosterone (DHT). Testosterone can also be converted to oestradiol through an aromatization process, but DHT cannot be converted to oestradiol (Larsson, 1979). In general, DHT is the active form of testosterone peripherally, causing cell proliferation in most tissues of the genital tract, and the aromatizable androgens are more active within the CNS. Of interest here is the fact that aromatase activity varies 1500-fold between different brain nuclei and 5α -reductase varies only 3-fold (Roselli et al., 1987) suggesting that the aromatase system has more specialised effects within specific neural loci. The importance of aromatization within the CNS is evidenced by the fact that the aromatization inhibitor, androst-1,4,6-triene-3,17-dione (ATD) blocks the

testosterone-induced mounting behaviour in rats when applied intracranially (Christensen & Clemens, 1975). However, if rats are treated with oestradiol at the same time as ATD and testosterone, sexual behaviour is activated (Sodersten et al., 1986), though these authors conclude that oestradiol may exert its influence on sexual behaviour by modifying androgen metabolism by the brain, rather than by acting as a direct stimulator of the behaviour itself. ATD has also been found to inhibit ultrasound production (a precopulatory behaviour) and copulation in male hamsters when administered peripherally (Floody & Petropoulos, 1987). It is also interesting that prenatal oestrogens, derived from neural aromatization of circulating androgens, appear to be important in the development of masculine sexual behaviour in the male ferret. It is thought that these oestrogens may sensitize the developing brain to the subsequent masculinizing action of testosterone shortly after birth (Tobet & Baum, 1987). This is particularly relevant as several species, including marmosets and humans, exhibit a postnatal surge in plasma testosterone levels (Dixon, 1986), the function of which is unclear at present though it may be involved in this neural masculinization. A further point is that castration appears to reduce aromatase activity in the POA of rhesus monkeys, but that 5 α -reductase activity is unaffected (Roselli et al., 1987). It therefore seems likely that the two major pathways of androgen metabolism in neural tissue (aromatization and 5 α -reduction) are differentially regulated and probably fulfill distinct functions, with aromatization playing a more major role in the modulation of sexual behaviour by androgens. However, there are many species differences and conclusive evidence that aromatization is required for the effects of testosterone upon sexuality in male primates is lacking at present. Since masculine sexual behaviour is so dependent on gonadal steroids and on the integrity of the MPOA-AH, it is possible that the effects of the

aromatized testosterone seen above are mediated within this neural area. In order to establish where a hormone acts, two lines of evidence are required. First, it must be demonstrated that locally applied hormones can effect the hormonally dependent changes in behaviour, and second, it should be established that hormones are actually accumulated at the site in question and that the relevant receptors are present (Barfield, 1979).

Regarding the first point, there have been a number of studies to investigate the effects of intracranial implants of gonadal steroids on the sexual behaviour of castrated males in the rat (Davidson, 1966; Johnston & Davidson, 1972), the chick (Gardner & Fisher, 1968), the dove (Hutchison, 1971; Barfield, 1971), the lizard *Anolis carolinensis* (Morgantaler & Crews, 1978) and the gerbil (Yahr et al., 1982). In all these studies, it was found that implants of testosterone propionate or free testosterone in the MPOA-AH were the most effective in stimulating a resumption of sexual activity. Placing testosterone implants in other intra- or extra-hypothalamic sites, or using DHT implants within the MPOA-AH, were not as effective.

The fact that the MPOA-AH contains receptors specific to testosterone, or more precisely, its metabolites, was demonstrated in a neat study by Michael et al. (1987). To locate those neurons in which nuclear oestrogen receptors are present, castrated rhesus monkeys were injected with [^3H]-oestradiol. Half of the monkeys had been previously treated with oil (controls) and half with testosterone propionate. Compared with the control animals, nuclear levels of [^3H]-oestradiol in testosterone-treated males were reduced by 93% in the MPOA. The authors conclude that this is because the oestrogenic metabolites of testosterone had previously bound to the receptors, thus preventing the uptake of [^3H]-oestradiol. The MPOA-AH is not the only neural area that contains androgen receptors, they are also found in the corticomedial amygdalae, pituitary, septum, hippocampus and other

hypothalamic regions (Michael et al., 1987; Prins et al., 1988), but it is thought that these MPOA-AH androgen receptors are particularly important in the control of masculine sexual behaviour.

It is interesting to speculate on the mechanism of action of androgens within the MPOA-AH on the control of sexual behaviour. When cordycepin, an adenosine analogue that preferentially impairs synthesis of polyadenylated mRNA, is infused into the MPOA of castrated male gerbils an hour before they receive systemic injections of testosterone propionate, it almost completely blocks recovery of sexual behaviour (Yahr & Ulibarri, 1987). This, coupled with the fact that cycloheximide (an inhibitor of protein synthesis) infused into the POA of intact male mice also inhibits sexual behaviour (Quadagno et al., 1976), suggests that androgens bind to nuclear receptors within the MPOA-AH thereby stimulating protein synthesis which, in turn, activates masculine sexual behaviour.

Androgens are also known to stimulate POA Na^+, K^+ - ATPase activity in male rats (Guerra et al., 1987). In this study, it was found that testosterone propionate treatment to castrated animals resulted in a 4-fold increase in enzyme activity in the preoptic-suprachiasmatic region, and this effect may be related to the behavioural effects of androgens.

A further insight into the mechanism of action of androgens comes from the fact that doses of testosterone propionate which are ineffective in inducing or maintaining male sexual behaviour in rats, are potentiated by concurrent administration of theophylline. This substance increases levels of cyclic AMP by inhibiting the enzyme which inactivates it, and this result may indicate a mediating role for cyclic AMP in the regulation of masculine sexual behaviour by testosterone (Christensen & Clemens, 1974).

2.1.3.4 Evidence for a role for the MPOA-AH in the hypothalamic-pituitary axis

It is possible that the MPOA-AH is exerting some of its effects on masculine sexual behaviour by modulating the release of gonadotrophins or other pituitary hormones. For this to be possible, it must first be determined whether the MPOA-AH contains LHRH-releasing neurons, and whether manipulations within the MPOA-AH do indeed have an effect on gonadotrophin secretion.

It is clear from anatomical studies that the MPOA-AH does contain LHRH-releasing neurons (Silverman et al., 1977; Silverman, 1984; Roselli et al., 1987). Further evidence comes more recently from a study in which POA grafts were transplanted into hypogonadal mice resulting in an increase in plasma LH and FSH levels, thus proving that these grafts contain LHRH-releasing neurons (Charlton et al., 1987).

There is also evidence to indicate that manipulations within the MPOA-AH do alter gonadotrophin secretion in some species. Firstly, acute electrical stimulation of the MPOA in male bullfrogs results in significant elevations in plasma LH and FSH. The pituitary response to this stimulation is rapid and of brief duration, and there is also a stimulus-response relationship. This pituitary response to electrical stimulation is similar in character to that induced by exogenous LHRH (McCreery, 1984). Secondly, it has been found that catecholamine release in the POA of ovariectomized rhesus monkeys is pulsatile and correlated with plasma LH levels. This pulsatility is largely reduced or abolished following oestrogen treatment, suggesting that the pulse may act as a signal character of the release of LH (Fuchs et al., 1986). This release pattern would appear to be independent of the pulsatile release of LHRH from the arcuate nucleus which also results in a rhythmical release of LH from the pituitary (Pohl & Knobil, 1982). Thirdly, implants of

cholecystokinin (CCK) in the POA in rats induce the release of gonadotrophins, but this release can be blocked by the dopamine receptor blocker, pimozide. This indicates that the facilitatory action of CCK on gonadotrophin secretion is mediated via dopamine receptors situated within the POA (Hashimoto & Kimura, 1986). A final piece of evidence for the modulation of gonadotrophin secretion by the MPOA-AH comes from a study which investigated the mechanism by which neonatal testosterone treatment alters the noradrenergic-opioid interaction which regulates LH secretion in adult female rats. The authors conclude that this is modulated via the presynaptic opioid input to the noradrenergic terminals situated within the MPOA (Grossman et al., 1987).

However, there does appear to be species variation as extensive lesions within the POA and AH of rhesus monkeys do not effect LH release (Plant et al., 1979).

As the MPOA-AH does modulate the hypothalamic-pituitary axis in some species, it is therefore feasible that some of its regulatory effects on masculine sexual behaviour are mediated via this route. However, the fact that the deficits caused by MPOA-AH lesions develop immediately post-operatively, are not reversed by exogenous androgens (see section 2.1.1) and that the testes do not atrophy (Brookhart & Dey, 1941; Hart et al., 1973), suggests that, although this may be the case, the MPOA-AH exerts its major influence on masculine sexual behaviour through other neural mechanisms.

2.1.3.5 Neurotransmitter systems within the MPOA-AH

As it is now clear that the MPOA-AH plays a crucial role in the control of masculine sexual behaviour, the next question that must be answered is which neurotransmitter systems are involved in this control.

(a) Opiates.

The inhibitory effects of opiates on sexual behaviour have been known for a long time, and the notion that they produce sensations in humans similar to sexual orgasm has been known since at least the eighteenth century, when a British physician wrote:

It has been compar'd, not without good cause, to a permanent gentle Degree of that Pleasure which Modesty forbids the name of.

John Jones, 1700.

However, it is only within the last decade that the effects of opioids on sexual behaviour have been studied extensively, and a number of hormonal and neurochemical correlates have been established.

Disturbances in sexual motivation and performance have been reported during heroin addiction and methadone maintenance (Cushman, 1972). These patients disclosed a high incidence of libido, potency and ejaculation difficulties accompanying heroin use, though those patients maintained on methadone reported an improvement in their sexual relationships. In a slightly later study, β -endorphin infused bilaterally into the ventricles of male rats resulted in deficiencies in mounting and intromissions (Meyerson & Terenius, 1977).

Since these studies, a number of investigations have been carried out using both opiate agonists and antagonists. Naloxone, a specific inhibitor of opioid receptors, induces sexual behaviour in sexually inactive male rats (Gessa et al., 1979) though not in sexually inactive male rhesus monkeys (Glick et al., 1982) and actually inhibited the sexual behaviour of socially reared, sexually active intact male monkeys (Abbott et al., 1984). Naloxone and naltrexone - another opiate antagonist - have also been reported to stimulate affiliative behaviour such as grooming and grooming invitations in talapoin monkeys (Fabre-Nys et al., 1982). The opiate agonist methadone,

has also been found to reduce sexual performance and motivation in the male Syrian golden hamster, presumably also by acting on specific opiate receptors (Murphy, 1981). It is thought that the slightly contradictory effects of opiate antagonists and agonists are mediated by the blockade of the actions of endogenous opioids at the cell opioid receptor (Abbott et al., 1984).

Recently, a study was undertaken to elucidate the site of action within the rat CNS at which opiates act in the control of masculine sexual behaviour. Sexual behaviour was inhibited following bilateral infusions of β -endorphin within the MPOA-AH, but not following infusions into the adjacent bed nucleus of the stria terminalis or rostral VmH (Hughes et al., 1987). This inhibition of sexual behaviour was prevented by either pretreating rats with naloxone intraperitoneally, or by infusing a putative delta opiate receptor blocker, ICI 174864, into the MPOA-AH 5 minutes before β -endorphin treatment.

There has also been some work carried out to elucidate how the opiate system impinges on the control of sexual behaviour. Opiate antagonists, naloxone and naltrexone, have been found to increase plasma LH and testosterone levels in the rhesus monkey (Abbott et al., 1984) and the talapoin monkey (Fabre-Nys et al., 1982) suggesting that endogenous opiates influence the hypothalamic-pituitary axis as well as sexual behaviour *per se*; and in fact there is now much evidence to implicate endogenous opiates in the control of pituitary function (Bicknell, 1985; Pfaus & Gorzalka, 1987). Two further points are of interest here. Firstly, saturable stereospecific binding of [3 H]-naltrexone in rat brain homogenates prepared from castrated males is greater than the corresponding binding in intact males though castrated males do not have higher hypothalamic β -endorphin levels (Hahn & Fishman, 1985); and secondly, diminished levels of β -

endorphin-like immunoreactivity and LHRH content are seen in the brains of aged male rats with impaired sexual behaviour (Dorsa et al., 1984). It is therefore possible that some of the effects of opiates on masculine sexual behaviour may be mediated via the hypothalamic-pituitary axis.

However, it is clear that not all (if any) of the effects seen are due to the disruption of this axis, as naloxone causes reductions in the frequency of intromissions preceding ejaculations and ejaculation latency even in hypophysectomized male rats (Myers & Baum, 1980).

There is evidence that penile erection may be partially controlled by an opiate-dopamine interaction (Berendsen & Gower, 1986) and it is clear that dopamine stimulates penile erection (see below).

One last piece of evidence that indicates the method of action of endogenous opiates in the control of male sexual behaviour comes from a study investigating central catecholamine levels following intraventricular infusions of β -endorphin (McIntosh et al., 1980). The administration of β -endorphin caused a complete loss of copulatory behaviour in male rats, and this deficit was correlated with a significant increase in hypothalamic noradrenaline levels - a neurotransmitter which has been associated with the inhibition of sexual behaviour (see below). The authors suggest that the endogenous opiates may be involved in the mediation of sexual behaviour via an interaction with central catecholamine systems.

(b) Monoamines.

i) Noradrenaline. Spinal cord concentrations of noradrenaline have been found to be elevated after intromissions and ejaculations in the male rat (Mas et al., 1987) and further evidence of the role for noradrenaline in reproductive function comes from work on the Japanese quail (Ottinger & Balthazart, 1987). In this study, the turnover rate of noradrenaline within the telencephalon of male quails was found to be significantly decreased by

castration which is coupled with sexual inactivity.

Pharmacological manipulations of noradrenergic systems have resulted in alterations of sexual behaviour. Malmnas (1973) reported that inhibition of noradrenaline synthesis by FLA-63 (bis(4-methyl-1-homopiperazinyl thiocarbonyl)-disulphide) increased the rate of mounting in castrated male rats treated with suboptimal doses of testosterone. However, in contrast, inhibition of noradrenaline synthesis in intact males with diethyl-dithiocarbamate was reported to have increased mount, intromission and ejaculation latencies, and lengthened the postejaculatory interval in male rats (McIntosh & Barfield, 1984c). Depletions of central noradrenaline, by DSP4 (N-2-chloroethyl-N-ethyl-2-bromobenzylamine - a specific noradrenergic neurotoxin) injections (Hansen et al., 1982b) or by electrolytic lesions of the locus coeruleus (McIntosh & Barfield, 1984c), similarly prolonged the postejaculatory interval. This later evidence suggests that noradrenaline exerts an stimulatory influence on masculine sexual behaviour.

ii) Dopamine. Dopamine was originally implicated in the control of masculine sexual behaviour through human studies on Parkinson's disease. It was found that in men suffering from this disease, treatment with L-dopa sometimes enhanced sexual activity (Bowers, et al., 1971).

In the male rat, disruption of central dopaminergic pathways by electrolytic lesions of the substantia nigra or by localised intracerebral injection of 6-hydroxydopamine (a specific neurotoxin for catecholaminergic pathways), or the administration of the dopamine receptor blocker, pimozide, results in a significant increase in the postejaculatory refractory period (McIntosh & Barfield, 1984b). The authors suggest that central dopaminergic pathways are involved in the motivational or arousal component of copulation and may be integral to the maintenance of a normal postejaculatory refractory period.

In addition, more recently, dopaminergic activity within the POA has been correlated with sexual arousal (Mas et al., 1987) and dopamine agonists have been found to facilitate penile erection, but not necessarily copulatory behaviour, in the rhesus monkey (D_2 agonist, LY163502; Davis et al., 1987) and in the rat (apomorphine and the D_2 agonist, LY171555; Melis et al., 1987). In the latter study, the paraventricular nucleus was found to be the most effective neural locus for stimulating penile erection in isolated male rats.

iii) Serotonin. This indoleamine was traditionally considered to exert an inhibitory influence on masculine sexual behaviour (Bitran & Hull, 1987). Evidence for this view comes, in part, from a study that investigated the role of central serotonergic pathways in the control of sexual behaviour in the male rat (McIntosh & Barfield, 1984a). In this study, disruption of central serotonergic systems was achieved by; (i) selective electrolytic lesions of the midbrain raphe nuclei, or (ii) localized intraventricular or intracerebral injection of a specific serotonergic neurotoxin, 5,7-dihydroxytryptamine (5,7-DHT). A third group of animals was tested for sexual behaviour following peripheral injection of *p*-chlorophenylalanine (PCPA), an inhibitor of serotonin synthesis. Both electrolytic and neurochemical lesions localized in the dorsal raphe nucleus produced a highly significant shortening of the ejaculation latency and the postejaculatory refractory period. Disruptions of serotonergic mechanisms following intraventricular injection of 5,7-DHT or systemic administration of PCPA also caused a significant reduction in the length of the refractory period.

In a second study, the serotonin precursor 5-hydroxytryptophan tended to suppress sexual behaviour whilst the serotonin antagonist, methysergide, elevated sexual activity in male rats (Lieblich et al., 1985), and, in a later study, the serotonin content of the POA was found to increase on ejaculation

in male rats (Mas et al., 1987), also indicating an inhibitory role for this monoamine.

However, more recent studies have recognised a number of subclasses of serotonin receptors, and it is now suspected that some of these receptor types exert a stimulatory influence on masculine sexual behaviour (Bitran & Hull, 1987).

(c) Adrenoceptors.

As the central monoamine systems appear to be involved in the regulation of sexual behaviour, it is important to determine which receptors are employed in this modulation.

α -adrenoceptors have been implicated in the behavioural, as well as physiological, processes controlled by the CNS. Central α -adrenoceptors are divided into two subtypes based upon pharmacological criteria. α_1 -adrenoceptors have only been found at post-synaptic sites whilst α_2 -adrenoceptors are predominantly located pre-synaptically. Also, electrophysiological studies indicate that α_1 -adrenoceptors mediate a facilitation of excitation, whereas α_2 -adrenoceptors mediate inhibition both pre- and post-synaptically. Both types of receptor have been identified in the POA and other hypothalamic areas (Clark et al., 1985).

Clonidine, a commonly used antihypertensive agent which is believed to act by stimulation of central α -adrenoceptors, produces a dose-related suppression of ejaculatory behaviour in sexually vigorous male rats (Clark et al., 1985) and impotence in some men during antihypertensive trials (Onesti et al., 1971; Mroczek et al., 1972). Prazosin, another hypertensive agent that acts by blockade of α_1 -adrenoceptors, increased latencies to initiation of copulation and ejaculation, and to reinstatement of copulation following ejaculation. Additionally, prazosin pretreatment fails to attenuate or prevent the clonidine-induced suppression of ejaculation. In contrast, yohimbine, a

drug which preferentially blocks α_2 -adrenoceptors, caused a facilitation of copulatory behaviour, as evidenced by drastic decreases in ejaculation latency and postejaculatory intervals. Pretreatment with yohimbine completely prevents the clonidine-induced suppression of ejaculation, whilst clonidine attenuates the facilitatory effects of yohimbine, suggesting a competitive interaction (Clark et al., 1984; Clark et al., 1985). Furthermore, methoxamine, an α_1 -adrenoceptor agonist, causes reductions in the ejaculatory threshold, evidenced by a decrease in the number of intromissions preceding ejaculation (Clark et al., 1987).

All this evidence leads to the suggestion that increased excitatory adrenergic activity results in increased sexual arousal either by blockade of α_2 - or stimulation of α_1 -adrenoceptors. Alternatively, stimulation of α_2 - or blockade of α_1 -adrenoceptors results in diminished sexual motivation.

(d) Peptides.

i) LHRH. In rats, LHRH will reduce mount and ejaculatory latencies provided that some testosterone is also present (Moss, 1978; Myers & Baum, 1980). Clinical studies of men also indicate that LHRH has direct central effects on potency (Mortimer et al., 1974; Mortimer et al., 1976). Although Benkert (1975) found no consistent effects of LHRH in impotent men when administered by nasal spray, some patients experienced an increase in erections after treatment was discontinued, and Evans and Distiller (1979) found that the rapidity of onset of erection, maximum degree of erection obtained, and overall levels of penile tumescence were greater following erotic stimulation in men that had received LHRH than in placebo controls, though the increases were not statistically significant.

Despite these studies, the role of LHRH in the control of masculine sexual behaviour remains unclear.

ii) Prolactin. This peptide has been extensively implicated as an inhibitor of

masculine sexual behaviour in rats (Bailey & Herbert, 1982; Weber et al., 1982; Kalra et al., 1983) and mice (Svare et al., 1979) and impotence is associated with hyperprolactinaemia in men (Perryman & Thorner, 1981). It is interesting to note that plasma levels of prolactin rise in sexually experienced rats following exposure to a mating arena (Kamel et al., 1975) and on ejaculation in mice (Bronson & Desjardins, 1982) indicating a possible role in controlling the post ejaculatory interval. There would appear to be a species difference in the effects of high prolactin levels on the hormonal response of males to female conspecifics and on copulatory activity. Both rats and mice normally exhibit an acute release of testosterone and LH on exposure to a female. Hyperprolactinaemic mice of certain strains continue to show this response and copulatory behaviour is maintained. However, hyperprolactinaemic rats no longer show this acute release of LH in response to a female (though their testosterone response is unaffected) and also show a loss of copulatory activity. This failure of hyperprolactinaemic male rats to experience an increase in plasma LH levels in response to a female suggests an abnormality in the mechanisms controlling LHRH release. Suppression of LHRH release may also be involved in the induction of the deficits in sexual behaviour seen in hyperprolactinaemic males (Sellers & Bartke, 1987).

Electrophysiological studies of the male rat have shown that refractory periods of some neurons projecting from the amygdala to the POA in the stria terminalis increase during chronic hyperprolactinaemia. The refractory periods of these neurons resemble those of castrated males, but normal concentrations of circulating testosterone can be maintained by implanting silastic capsules of hormone subcutaneously into hyperprolactinaemic males (Kendrick & Dixon, 1984c). Thus chronic hyperprolactinaemia may impair sexual behaviour by decreasing central sensitivity to testosterone.

iii) Oxytocin. Plasma levels of oxytocin in both males and females are elevated during and immediately after copulation in cattle (Sharma & Hays, 1973), rabbits (Stoneham et al., 1985; Todd & Lightman, 1986), sheep (Sharma et al., 1972; Garcia-Villar et al., 1985), goats (McNeilly & Ducker, 1972) and humans (Fox & Knaggs, 1969; Ogawa et al., 1980; Carmicheal et al., 1987), genital stimulation in bulls (Peeters et al., 1983) or vaginocervical stimulation in goats (Seckl & Lightman, 1987).

Peripheral injections of oxytocin have been found to alter the characteristics of rabbit semen, causing an increase in the volume of the ejaculate (Fjellstrom et al., 1968), though oxytocin does not appear to significantly affect plasma testosterone levels (Tan & Kwan, 1987).

There has been very little work carried out on the central action of oxytocin in the control of sexual behaviour, though preliminary studies would suggest that it exerts an inhibitory influence. For example, infusion of oxytocin into the third ventricle of male rats increased the latencies to the first mount and intromission and lengthened post-ejaculatory refractory periods (Stoneham et al., 1985).

Interestingly, a later study indicated that intracerebroventricular administration of oxytocin induces penile erection in male rats though, in this study, males remained isolated from females (Argiolas et al., 1986). In an attempt to localise this central action of oxytocin, infusions were made into the paraventricular nucleus, hippocampus, lateral septum, caudate nucleus, subiculum, POA, VmH and supraoptic nucleus. Only infusions into the paraventricular nucleus or the hippocampus resulted in penile erection in rats (Melis et al., 1986).

iv) Vasotocin. Sexually responsive male rough-skinned newts have significantly higher levels of arginine vasotocin in various neural areas, including the POA, compared with sexually unresponsive males (Zoeller &

Moore, 1988). The authors conclude that endogenous arginine vasotocin has a stimulatory effect on masculine sexual behaviour.

v) Corticotropin-releasing factor - CRF. Acute microinfusions of ovine CRF into the third ventricle of sexually experienced male rats produce a dose-dependent suppression of masculine sexual behaviour that can be reversed by simultaneous infusions of naloxone. These findings suggest that CRF can exert effects on male reproductive behaviour, and that these actions may be mediated through mechanisms which involve the activation of opioid pathways within the CNS. It is also possible that this CRF-linked neurochemical signal may mediate some of the well-known deleterious effects of stressful and noxious stimuli on reproductive function (Sirinathsingji, 1987).

The related peptides, adrenocorticotrophin (ACTH), melanocyte stimulating hormone, and β -lipotrophin all contain a series of seven amino acids (ACTH 4-10) which exerts extraordinary effects upon sexual reflexes in male rabbits. Intraventricular infusion of these peptides results in repeated spontaneous erections and ejaculations. These responses begin after 15 minutes and males do not attempt to mount females (Bertolini et al., 1975).

2.1.4 The connections of the MPOA-AH

The importance of the MPOA-AH in the control of masculine sexual behaviour has stimulated interest in the pathways that connect this area to other brain structures which may also be involved. Two major techniques have been applied in behavioural research; brain lesions (electrolytic or neurotoxic) along the course of a fibre tract, or knife cuts. The two most important pathways involved in the control of sexual behaviour are the major

efferent pathway of the MPOA-AH - the medial forebrain bundle, and the major afferent connection - the stria terminalis. However, it is important to remember that, although the majority of fibres within each tract project in the direction stated, both pathways contain a smaller number of fibres travelling in the opposite direction. It is possible that these fibres might also play a part in any effects seen following mechanical damage to a particular fibre tract.

2.1.4.1 The medial forebrain bundle (MFB)

In male rats, lesions in the MFB in the anterior, medial and posterior hypothalamus severely disrupt copulatory activity (Hitt et al., 1970; 1973). The fact that lesions in more than one anteroposterior location in this tract have the same effect on behaviour indicates that these effects are due to the interruption of a pathway rather than to the destruction of neurons within a discrete area. It has been found that lesions at different points along the MFB have varying effects on behaviour. MFB lesions in the medial hypothalamus and those extending above the MFB in the posterior hypothalamus are more effective in disrupting copulation than ventrocaudal lesions, and the severity of the copulatory deficit is significantly related to telencephalic noradrenaline depletion. The results suggest that, as the posterior hypothalamus is approached, a significant proportion of noradrenaline fibres responsible for producing the copulatory deficit leave the MFB in a dorsal direction (Caggiula et al., 1973).

MFB lesions interfere with the initiation but not with the execution of copulation as lesioned rats either fail to copulate or copulate after prolonged first mount and intromission latencies. However, once the first copulatory response is achieved in a given test, ejaculations follow at the normal rate (Szechtman et al., 1978). Furthermore, the deficit caused by such lesions is not due to reduced responsiveness to general arousal as replacing the

female, handling, and tail shock, which normally produce behavioural arousal, still fail to induce copulation (Caggiula et al., 1974).

The fact that it is the connections of the MPOA-AH with the MFB that are of particular importance is clear from studies that employ a knife-cut technique to sever these connections. Parasagittal knife cuts that extend from the POA through the ventromedial nucleus separating mediolateral connections, severely disrupt copulatory behaviour, whereas cuts anterior to and immediately caudal to the MPOA-AH do not disturb mating activity (Paxinos & Bindra, 1972; Szechtman et al., 1978).

Additional evidence that the impairment in sexual behaviour seen following MFB damage is due to its connections with the MPOA-AH has been given by Hendricks and Scheetz (1973). They found that unilateral MFB lesions coupled with contralateral MPOA-AH lesions resulted in impairment of male sexual behaviour comparable to that seen following bilateral MPOA-AH lesions. A further point of interest here are the results obtained from another study in which an asymmetrical lesioning technique was also employed (Brackett & Edwards, 1984). Efferents from the MPOA course through the MFB to pass through and/or terminate in the dorsolateral and ventral tegmentum of the midbrain and, in this study, it was found that bilateral lesions of the dorsolateral tegmentum eliminate mating behaviour in male rats, reproducing the effects of MPOA-AH lesions. Sexual behaviour was also abolished when a POA lesion on one side was coupled with a contralateral dorsolateral lesion, suggesting that the integrity of the pathway between these two structures via the MFB is essential for the maintenance of copulatory behaviour. However, a study using neurotoxic lesions within the dorsal midbrain tegmentum in rats reports contradictory results of a post-operative acceleration of mating, primarily by reducing the post ejaculatory interval (Hansen et al., 1982b). However, when only the peripeduncular



nucleus within the dorsal midbrain tegmentum was destroyed with ibotenic acid, ejaculatory behaviour was almost completely abolished, though the males continued to mount (Hansen & Kohler, 1984).

One final piece of evidence for the involvement of the MFB comes from another study (Hansen et al., 1982a) in which neurotoxin was also infused into the area lateral to the MPOA-AH (where electrolytic and thermal lesions have been found to cause copulatory deficits) and no deficits were seen, indicating that the results from traditional lesion studies are indeed due to the disruption of the MFB fibre connections.

2.1.4.2 The stria terminalis (ST)

There is much evidence to implicate the entire pathway from the olfactory bulbs via the corticomedial amygdala and ST to the MPOA-AH in the control of masculine sexual behaviour. The evidence implicating the other neural structures within this pathway is discussed in later sections and the account below is limited to the ST.

Giantonio et al. (1970) found that lesions in the ST of rats resulted in an increase in the time required to achieve ejaculation i.e. the time from the initial mount with thrusting in a series to an ejaculation, and a slight reduction in the number of ejaculations to exhaustion, suggesting a role for the ST in the control of copulatory activity. However, these relatively moderate effects on copulatory behaviour are disputed by a study in which knife cuts in the ST caused severe deficits in sexual activity (Paxinos, 1976). The suggestion that the neurons projecting via the ST influence copulatory activity rather than sexual arousal is in agreement with further studies in which it was found that the MPOA-AH influences the achievement of ejaculation and the subsequent refractory period through its dorsal connections with the ST (Szechtman et al., 1978), and that the ST influences

the temporal patterning of copulation (Lehman et al., 1983).

Lesions within the bed nucleus of the ST also influence sexual activity. Male rats receiving these lesions displayed more intromissions preceding each of three ejaculations in a test, and also tended to be slower in the temporal pattern of copulation (Emery & Sachs, 1976). A similar result has recently been reported following lesions in the bed nucleus of the ST of male hamsters (Powers et al., 1987).

2.1.5 Other areas of importance

Although the MPOA-AH plays a fundamental role in the control of masculine sexual behaviour, there are other neural areas that are of equal importance. The layout of this review does not mean to imply that the MPOA-AH is the integrative centre as it is not known how the neural structures are related in a hierarchical manner and furthermore, the relative importance of each area may vary between species.

The other major neural areas that have been implicated in the control of masculine sexual behaviour are discussed in turn.

2.1.5.1 The olfactory system

In terms of the involvement of the special senses in sexual behaviour, the olfactory system has received the most attention. The reason is obvious, males of many species spend considerable time smelling the genitalia of females or urine eliminated by them. In all nonprimate mammals studied, males are able to discriminate by olfaction between receptive and nonreceptive females. However, the visual system is also brought into play in detecting species-typical receptive stances and behaviours of females

and, in some species such as cats and frogs, mating calls attract the two sexes to one another. It has been known for a long time however that, for rats and rabbits at least, none of the special senses are indispensable for the expression of male sexual behaviour (Hart & Leedy, 1985). Nevertheless, in some species such as the hamster, olfaction is vital (Murphy & Schneider, 1970). Furthermore, the MPOA-AH receives major neural inputs from the olfactory system (Scalia & Winans, 1975) so, for these reasons, its role in the control of sexual behaviour in males of some species is discussed below.

Most of the studies designed to explore the effects of anosmia on male sexual behaviour have involved the production of anosmia by peripheral means, which may or may not eliminate accessory as well as main olfactory function, or the production of anosmia by olfactory bulbectomy, which eliminates the function of both chemoreceptive systems. In general, these procedures have little effect on sexual behaviour in the laboratory situation. Peripheral anosmia does not impair the copulatory performance of sexually experienced dogs (Hart & Haugen, 1971), owl monkeys (Dixson, 1983b) or rhesus monkeys (Goldfoot et al., 1978) and, in cats (Aronson & Cooper, 1974) and sheep (Fletcher & Lindsay, 1968), olfactory bulbectomy does not impair sexual activity in sexually experienced animals. In male rats, there is some disruption of sexual activity following bulbectomy, especially if the animals are sexually naive before the operation is performed. In this case, olfactory bulbectomy results in longer intromission and ejaculation latencies and a lowered probability of ejaculation on each test (Larsson, 1979). Lesions in the olfactory tubercle also appear to produce decrements in the sexual behaviour of male rats (Hitt et al., 1973) but, unfortunately, in this latter study the sham-operated controls also showed a reduction in sexual activity post-operatively confusing any clear interpretation of the data. In contrast, copulatory behaviour is virtually eliminated in male hamsters

following olfactory bulbectomy (Murphy & Schneider, 1970; Winans & Powers, 1974).

However, the fact that in most species, totally anosmic males mate under laboratory conditions, does not mean that olfaction or olfactory bulb neural activity is unimportant in the expression or development of sexual behaviour under more natural conditions. Oestrogen stimulates and progesterone depresses female sexual attractiveness in rhesus monkeys by affecting the vagina (Baum et al., 1976) and much attention has been focused upon the role of vaginal odour in this context, though the reports are conflicting. Michael and Keverne (1968) used an operant conditioning paradigm to show that male rhesus monkeys rendered temporarily anosmic showed increased bar-pressing activity to gain access to oestrogen-treated females once olfactory ability was reinstated. In contrast, Goldfoot et al. (1978) showed that 3 anosmic males continued to show rhythmic changes in ejaculatory frequency during their female partners' menstrual cycle though, in this latter study, the males may have been substituting other sensory or behavioural cues for olfactory ones.

Michael and Keverne (1970) found that lavages obtained from ovariectomised oestrogen-treated females enhanced sexual attractiveness when applied to the rumps of untreated females, and it has since been discovered that the active portion of the lavage consists of various aliphatic acids formed, in part, by microbial action on vaginal secretions (Curtis et al., 1971; Michael et al., 1972). Vaginal secretions from female anubis baboons collected throughout a number of menstrual cycles cause rhythmical changes in male rhesus monkey activity with a female rhesus partner treated with the baboon secretions. The maximum mounting activity corresponds to the time of maximal sexual swelling of the female baboon from which the collections had been made, thus indicating the similarities between the

olfactory cues from females of 2 different primate species (Michael et al., 1972). However, Goldfoot et al. (1976) could not demonstrate an effect of vaginal lavages from oestrogen-treated females on ovariectomised animals. A further problem in interpreting the influence of vaginal olfactory cues is that aliphatic acid levels actually increase during the luteal phase of the cycle, at a time when sexual interactions are usually decreasing in rhesus monkeys (Michael & Bonsall, 1977).

There is clearly inter-species variation in the relative importance of olfaction and, interestingly, it is the macrosmatic rodents which show deficits in sexual behaviour during anosmia though the full importance of olfaction in primate sexual behaviour is still unclear.

2.1.5.2 The amygdalae

Beginning with the first descriptions of the effects of temporal lobe ablations by Kluver and Bucy (1937), or more limited lesions restricted to the amygdaloid nuclei (Schreiner & Kling, 1956), a syndrome of behavioural changes (the Kluver-Bucy syndrome) resulting from such ablations in monkeys and cats can be characterized as follows; (i) a marked decrease in agonistic behaviour and a reduction of fear towards normally fear-inducing objects including man, (ii) hyperorality, including coprophagia and uroposia, (iii) 'hypermetamorphosis', or an excessive tendency to attend to and react to every visual stimulus, and (iv) increased and inappropriate sexual behaviour (Kling, 1974). These behavioural changes can also be induced in man by the bilateral removal of the temporal lobes and underlying amygdalae (Terzian & Dalle Ore, 1955; Shraberg & Weisberg, 1978) and hypersexual episodes are sometimes seen in temporal lobe epilepsy (Blumer, 1970).

From these early studies, it was thought that the amygdalae exert an inhibitory influence on sexual behaviour in both sexes, possibly via the AH.

However, although amygdaloid lesions result in hypersexuality in primates and cats, lesions within the anterior amygdalae in rats have no effect on sexual behaviour and lesions within the basomedial-corticomedial amygdalae actually produce an increase in ejaculation latencies and a slight decrease in the number of ejaculations to exhaustion (Giantonio et al., 1970). In a later study, corticomedial amygdaloid lesions in rats were again shown to reduce copulatory activity on the first post-operative test, although recovery then occurred (Harris & Sachs, 1975). In this study, changing the female partner or pinching the tail of the male was found to stimulate copulation suggesting that a deficit in arousability may be involved.

In a more recent study, it was argued that 'hypersexuality' is not the correct definition of the behaviour seen following amygdalectomy, and 'inappropriate sexual behaviour' would be a better description. Male cats were lesioned in the basolateral nuclei of the amygdalae and given normal pair tests and also selection tests during which they were offered a soft toy, a moving toy, a tranquilized rabbit, a tranquilized male and an oestrous female. In these selection tests, the major post-operative change was an increased tendency to mount inappropriate objects but there was no evidence of hypersexuality in the sense of a higher level of sexual performance (Aronson & Cooper, 1979). This change was not interpreted as the description of an amygdaloid mechanism directly regulating sexual activity, but rather as interference with a system which normally modulates approach and withdrawal behaviour in response to various stimuli.

The theory that the amygdalae regulate general social interactions and emotional responses to other individuals and objects, rather than purely sexual behaviour also gains support from the work on amygdalectomy in rhesus monkeys which are living in social groups. In this situation, three main effects are seen, (i) the lack of maternal behaviour by lesioned

mothers, (ii) a tendency towards reduced social interactions and a decrease in social rank, and (iii) social isolation within free-living groups (Kling, 1974).

2.1.5.3 The neocortex

The neocortex plays an obvious role in masculine sexual behaviour apart from the general sensory and motor functions necessary for any species-typical behaviour. Various types of early experience and heterosexual contacts have been shown to have profound influences on male sexual behaviour. For example, male dogs raised in semi-isolation, having little contact with other dogs, show sexual excitement as adults in the presence of oestrous females but the behaviour is characterized by a high frequency of incorrectly orientated mounts (Hart & Leedy, 1985). Also, sexually experienced male rats usually suffer less impairment of mating performance than sexually inexperienced rats after olfactory bulbectomy (Larsson, 1979). It is logical to assume that the cerebral cortex plays a role in these and other experiential aspects of sexual activity. The tendency has been to ascribe to the neocortex functions related to arousing or maintaining sexual excitement or interest. In addition, it has been argued that, in mammals with more highly evolved brains, the neocortex is more important in the overall mediation of sexual behaviour than in species with less developed brains (Beach, 1958). Complete removal of the cerebral cortex abolishes masculine sexual behaviour in male rats and cats but not rabbits, and temporary functional decortication by application of 25% KCl solution to the neocortex blocks sexual behaviour for a few hours (Larsson, 1979). According to Beach (1940), smaller lesions including less than 20% of the cortex did not impair sexual behaviour, whereas lesions including 60% of the total eliminated sexual behaviour in rats. The size, and not the position, of the lesions was thought to be the important factor. However, Larsson (1979), using fairly

small lesions (12% of the cortex), found that lateral lesions were more effective in abolishing male rat sexual behaviour than lesions of the medial parts of the cortex. Even more effective were lesions in the frontal pole - 8 out of 20 frontally lesioned rats stopped copulating post-operatively - though, in a later study, lesions of the orbitofrontal prefrontal cortex in rats were ineffective (De Bruin et al., 1983).

In cats, removal of the frontal lobes modifies the copulatory response, causing a prolonged mount latency, an increase in the proportion of mounts not culminating in intromission, and a decrease in the number of intromissions. Ablation of the temporal and parietal areas leave the behaviour unchanged, and occipital damage does not cause disturbances unless it is extensive enough to prevent the male from locating and following the female. Thus, in the cat, extensive frontal lesions cause a serious motor disability whereas, in the rat, comparable lesions do not result in any deviations in general motor performance but rather in a lack of sexual motivation (Larsson, 1979).

Cells in the inferotemporal cortex of macaques respond specifically to complex visual stimuli such as faces, and certain cells respond preferentially to changes in orientation of the face or changes in facial expression (Perrett et al., 1985; Kendrick & Baldwin, 1987). Facial communication plays an important role in recognition, particularly among primates, and in the co-ordination of sexual activity. Thus, 'eye-contact proceptivity' is an important prelude to copulation in the chacma baboon (Bielert, 1986). It is therefore possible that this area of the neocortex is particularly important in the control of sexual behaviour in species such as primates that rely heavily on visual cues.

One problem with interpreting results of cerebral cortex ablations is that subcortical structures can be invaded by the lesions and may themselves be

the neural substrate that causes the effects. This problem is particularly clear in the case of the Kluver-Bucy syndrome in which it appears to be damage to the amygdalae and not the overlying temporal lobes that cause the syndrome to develop.

2.1.5.4 The dorsomedial (DMH) and posterior (PH) hypothalamus

The involvement of the PH in the control of masculine sexual behaviour was first indicated by an intracranial stimulation study in rats (Caggiula, 1970). Both stimulation-bound copulation and bar pressing for the opportunity to copulate were produced by stimulation of hypothalamic sites. This stimulation was also rewarding and reward level (self-stimulation rate) decreased after castration and increased with testosterone injections. In a later study on rhesus monkeys, stimulation of the PH and DMH produced mounts of longer duration, with more thrusts per mount, higher thrusting rates and a greater number of ejaculations per test as compared to spontaneous sexual activity. Also, refractory periods between successive ejaculatory episodes were shortened as a result of stimulation-induced mounting behaviour (Perachio et al., 1979).

Intracranial recording techniques have also implicated the DMH and PH in the control mechanism. Neuronal activity in the DMH and PH shows specific changes during mounting, intromission and thrusting in monkeys (Yoshimatsu, 1983), and the author concludes that these areas are involved in the control of copulation and not of sexual arousal, which is in agreement with the data from the stimulation studies.

2.1.6 Penile erectile mechanisms

This section investigates the effects of damage to the spinal cord on penile erection and sexual behaviour and the effects of interfering with the pathways between the cord and peripheral structures. However, it is with a brief introduction to the anatomy, mechanics and innervation of the penis that this section begins.

The general plan of the penis is common to all mammalian species. The erectile bodies of the penis consist of the paired corpora cavernosa and the corpus spongiosum. The penile corpora are composed, in part, of smooth muscle and vascular tissue, and each of the corpora is more or less paired in its function with a set of striated muscles - the bulbospongiosus muscle which is the origin of the corpus spongiosum and the ischiocavernosus muscles which are continuous with the corpora cavernosa. A bone (os penis) may lie centrally in the body or glans of the penis (Dixson, 1987).

The mechanical basis for erection in most species lies in a combination of vascular, smooth muscle and striated muscle actions, although the relative contribution of each of these effector systems varies between species. The intracorporal actions involve the relaxation of the smooth muscles, allowing their expansion and an increased inflow of blood filling the corporal interstices. Co-ordinated with these vascular actions are those of the perineal muscles - the retractor penis, a smooth muscle that is tonically contracted, relaxes allowing extension of the penis (Sachs & Meisel, 1988).

The penis, its striated muscles and many of its accessory tissues are innervated by one or more of the pudendal, pelvic or hypogastric nerves. These nerves comprise the major efferent pathways for the regulation of penile erection. The dorsal penile nerve (DPN), a strictly sensory branch of the pudendal nerve, is the sole identified route of tactile sensory information

from the penis, although other genital afferent fibres are carried to the spinal cord by the pelvic nerve (Dixon, 1988; Sachs & Meisel, 1988). However, it should be remembered that most of the penile nerves carry both afferent and efferent fibres, making the relative contribution of each difficult to assess.

Systematic observations on human patients revealing that erection could be evoked in paraplegic and quadriplegic individuals date back 40 years to the post-war period (Munro et al., 1948) though work has also been carried out on casualties of more recent conflicts (Comarr & Gundersen, 1975). One conclusion from this later work, and from studies on laboratory animals, is that erection is easily evoked in spinal individuals, in which the spinal cord has been transected, but ejaculation is uncommon. Only in the dog have ejaculatory contractions and seminal expulsion been regularly elicited by stimulation of chronically maintained spinal subjects (Hart & Leedy, 1985). It is therefore clear that spinal reflexes are involved in the erectile response, but it would appear that higher neural centres are necessary for the development of the full copulatory performance culminating in ejaculation. A post-ejaculatory refractory period (5-30 minutes) occurs in these spinal dogs and therefore the refractory period seen following ejaculation in normal males may be partly due to changes at the spinal level.

Extensive uptake of dihydrotestosterone by the spinal cord in rhesus monkeys has been demonstrated by Sheridan and Weaker (1981) who postulate a role for androgens in the control of sexual reflexes in primates. This seems likely in view of Hart's studies on male rats and beagles which showed that testosterone facilitates sexual reflexes in the spinal male, and that these effects are not due to changes in penile morphology (Hart, 1967; 1968; 1973). In the rat, a sexually dimorphic motor nucleus occurs in the spinal cord between the fifth and sixth lumbar segments. This nucleus is larger in the male, accumulates tritiated testosterone and

dihydrotestosterone, and is known to innervate the penis (*bulbo cavernosus* muscles) and the *levator ani* muscle (Breedlove & Arnold, 1980, 1981). Dihydrotestosterone causes a prompt stimulation of penile erection in castrated rats, whereas oestradiol is ineffective (Gray et al., 1980) and dihydrotestosterone enhances the ability of oestradiol to restore sexual behaviour after castration in the rat (Larsson et al., 1973). It is possible that these effects are due to actions of androgens upon spinal sexual reflexes rather than to some synergism within the brain itself (Dixon, 1983a).

Descending monoaminergic systems within the spinal cord have also been implicated in the control of masculine sexual behaviour as administration of lisuride - a dopamine and serotonin agonist - into the subarachnoid space at the lumbar level markedly facilitates sexual behaviour in male rats (Hansen, 1982b).

The effects of penile deafferentiation have been studied in 2 ways - the transection of peripheral nerves and by application of topical anaesthetics to the penis.

Among the earliest extensive tests of penile denervation were those of Aronson and Cooper (1968) who severed the dorsal penile nerve (DPN) in cats. The effects of DPN transection on the cats were immediate and dramatic. Most of the males never again achieved intromission, though this was not due to the absence of penile erection, but because the attempted mounts of the males were severely disorientated.

In contrast with the effects on cats, severing the DPN of rhesus monkeys did not result in complete loss of the ability to achieve intromission. In successive operations, Herbert (1973) progressively removed more of the DPN. Behaviour continued to appear normal with up to 50% of the nerve removed bilaterally. With further nerve excision there was increasing disruption of the spatiotemporal characteristics of intravaginal thrusting until,

with complete removal of the nerves, thrusting was grossly abnormal. Nonetheless, even with all the DPN removed, the males continued to mount with normal orientation, had normal penile erections, and continued to gain intromission, albeit on a smaller proportion of mounts.

The common marmoset appears to have some effects in common with cats and some in common with rhesus monkeys. Transection of the DPN in male marmosets leads to more frequent mounting and a prolonged initial mount in each test. Males continue to orientate correctly during mounts and to make rapid pelvic thrusts, with the erect penis close to the vaginal orifice. However, the deep pelvic thrust required to attain intromission is abolished (Dixon, 1988).

It therefore appears that the sensory information carried by the DPN is not essential for the initiation or maintenance of penile erection, though it is necessary for the expression of full copulatory behaviour.

Several studies have also investigated the effects of pudendal nerve transection though this nerve innervates the striated penile muscles as well as receptors in the skin of the penis and peripenile areas making it difficult to attribute the effects to sensory or motor loss unambiguously. Lodder and Zeilmaker (1976) administered 4 mating tests to male rats in the 10 days following pudendal nerve transection, an interval short enough to preclude reinnervation of the target structures. In the first 3 tests, none of the 12 operated males achieved intromission or ejaculation. However, the latency to the first mount was unaffected by surgery, as was the temporal patterning of mounting clusters. These data reveal no decrement in sexual arousal during the post-operative period. Erection of the glans penis was absent during mounting in the first post-operative test but recovered gradually over the 10 day period. This pattern of results suggests that the pudendal nerve of the rat is necessary for erection and, perhaps secondarily, for intromission

and ejaculation.

Penile desensitization in male marmosets by application of a local anaesthetic (lidocaine) caused similar effects to transection of the DPN. Sexual arousal and erectile capacity were unaffected, though intromission latencies increased and 7 out of 10 males failed to intromit during some post-lidocaine tests (Dixson, 1986).

Some work has also been carried out on the role of the striated penile muscles in the control of erection, particularly in rats. Without the ischiocavernosus muscles, the male rat is virtually unable to achieve intromission, evidently because the penile body does not straighten enough to achieve the necessary proximity of the glans to the vaginal opening. In contrast to the effects of ischiocavernosus excision, removal of the bulbospongiosus muscle, including or excluding the dorsal portion (levator ani) does not alter any measure of copulation, nor does it prevent ejaculation of a plug of normal weight with a normal sperm count. However, males lacking the ventral bulbospongiosus muscle do not place the plug effectively against the cervix, thereby reducing sperm transport and the potential for pregnancy in mated females. Such operated males are also less effective at dislodging or removing plugs deposited in the female during previous matings. This handicap is probably due to the reduced ability of operated males to erect the spines on the surface of the glans, which would otherwise grip and dislodge the plug during the withdrawal phase of intromission (Sachs & Meisel, 1988).

2.2 Parental behaviour

2.2.1 Maternal vs. paternal behaviour

Most of the work carried out on the neural control of parental behaviour has involved rats. Much of this research has exploited the fact that both male and female rats can be induced to exhibit parental behaviour towards foster pups after a period of pup exposure. This phenomenon is known as sensitization or concavitation and takes 5 - 7 days in the case of a virgin female.

Much of this type of work has investigated the role of hormones in the initiation of maternal behaviour at parturition. It is clear that the hormonal changes coincident with parturition do facilitate the onset of maternal activities, as cross-transfusing blood from parturient mothers to virgin rats facilitates sensitization (Terkel & Rosenblatt, 1972), and the latency to sensitization can be reduced by imposing the hormonal changes characteristic of parturition on ovariectomised nulliparous rats (Moltz et al., 1970).

It is still not clear which hormones actually stimulate the onset of maternal behaviour at parturition, though it is generally agreed that oestrogen (Siegal & Rosenblatt, 1975a; 1975c; Rosenblatt & Siegal, 1975) and prolactin (Lamb, 1975; Rosenblatt et al., 1985) stimulate, whilst progesterone (Siegal & Rosenblatt, 1975b) inhibits this behaviour. This would seem logical as both oestrogen and prolactin levels rise at parturition and progesterone levels, which are high throughout pregnancy, fall around the time of birth.

However, it is obvious that the parental behaviour shown by males of some species cannot be under this same hormonal control system, but hormones

do appear to play a role in some aspects of paternal care in at least some species. Thus, plasma prolactin levels increase dramatically whilst male marmosets are carrying their offspring (Dixon & George, 1982), and male frogs that are exhibiting paternal care have significantly lower androgen levels than nonparental, mate calling, or mating males, and this decline in circulating androgens occurs during less than 12 hours of paternal care (Townsend & Moger, 1987). Furthermore, prenatal stress has been found to enhance maternal-like behaviour in male rats and is thought to exert its influence by altering the endocrinological milieu during the early stages of sexual differentiation (Kinsley & Bridges, 1988a).

There is clearly a non-hormonal parental behaviour control mechanism, as is evidenced by sensitization studies on virgin rats that receive no hormonal treatment (Rosenblatt, 1967). This control mechanism is generally considered to be neural, and has also been shown to be present in males, even in males of species that do not normally exhibit paternal care. For example, aspects of maternal-like behaviour are expressed by male rats following sensitization in the same way as by virgin females, though the process normally requires a longer period of pup exposure (Rosenblatt, 1967). Interestingly, this sensitization can be enhanced by high plasma levels of prolactin in juvenile male rats (Kinsley & Bridges, 1988b).

If paternal behaviour can be induced in males of species that do not normally show paternal care, the neural circuitry necessary for the expression of this behaviour is presumably present, but is normally inhibited, possibly via a hormonal mechanism (either high androgen levels or low prolactin levels), or via other neural circuits. It is therefore possible that, in species that do show varying degrees of paternal care, these same neural circuits are somehow activated and that they may parallel the neural mechanisms underlying parental behaviour in the female.

All but one of the studies on the neural control of parental behaviour in mammals have been carried out on the female and, in many of these, the MPOA has been established as a vital part of the neural circuitry.

2.2.2 The MPOA

2.2.2.1 Evidence for its importance in the control of parental behaviour

One of the earliest studies to implicate the MPOA in the control of parental behaviour was the only study in which males have been used. Aspects of maternal-like behaviour were elicited in male rats following intracranial implantation of sodium testosterone sulphate, and the most effective locus was the MPOA (Fisher, 1956). However, there are some criticisms of this study; only 5 out of more than 125 males showed aspects of maternal-like behaviour following this treatment, and the relevance of stimulating parental behaviour in male rats, which do not show paternal care under natural conditions, is questionable. The study does indicate an important fact however, the neural circuitry underlying parental behaviour appears to be present in males, even those of species that do not normally exhibit paternal behaviour.

Unfortunately, all subsequent studies have been carried out on females. In postpartum lactating female rats, MPOA lesions severely disrupt all aspects of maternal behaviour (Numan, 1974). In this study, no retrieval or nest building was seen in any of the females (n=10), and only 1 female ever adopted a nursing posture over her young and this only occurred once. Surgery was carried out on the 5th day postpartum, once maternal behaviour had been initiated, indicating that the MPOA is essential for the maintenance of maternal behaviour.

However, the MPOA also plays a role in the onset of maternal behaviour in virgin rats, as evidenced by a later study (Numan et al., 1977). In this study, none of the females that received MPOA lesions exhibited the full complement of maternal behaviours on exposure to pups, and those components that did occur, did so with significantly longer latencies than in sham-operated controls.

In these two studies relatively large lesions, encompassing most of the MPOA, were employed. Smaller lesions also disrupt maternal behaviour (Jacobsen et al., 1980). In this study, small bilateral electrolytic lesions in the MPOA of lactating female rats abolished nest building and retrieving components of maternal behaviour whilst crouching and nursing were unaffected. Animals which failed to show retrieval and nest building behaviours tended to have a greater area of lesion within the more dorsal part of the MPOA. The authors conclude that the dorsal MPOA plays a role in the maintenance of the active components of maternal behaviour in particular, but another critical factor in determining which components of maternal behaviour are disrupted may be the size of the lesion. As one increases the area of damaged tissue, there is also an accompanying increase in the components of maternal behaviour which are disrupted.

It would appear that protein synthesis within the POA is important in the maintenance of maternal behaviour as cycloheximide, a protein synthesis inhibitor, infused bilaterally into the POA of female rats on the fourth day postpartum, significantly suppresses maternal behaviour for 72 hours (Ho et al., 1974).

2.2.2.2 The importance of the central action of hormones within the MPOA

As steroid hormones and the MPOA appear to be so important in the control of maternal behaviour and the MPOA contains steroid receptors (see section

2.1.2.3), it is interesting that oestradiol benzoate (EB) implants within the MPOA facilitate the onset of maternal behaviour in the 16 day pregnant, hysterectomised and ovariectomised female rat (Numan et al., 1977). Such rats, when given EB implants in the MPOA, have significantly shorter latencies for the onset of maternal behaviour than have females implanted with cholesterol in the MPOA or with EB in the ventromedial hypothalamus, mammillary bodies, or under the skin.

A second study investigated the effects of MPOA EB implants on the maternal behaviour of ovariectomised nulliparous rats (Fahrbach & Pfaff, 1986). Again, EB implanted animals required significantly shorter exposures to pups than did cholesterol-treated controls before initiating carrying and grouping of dispersed pups in a maternal nest.

2.2.3 The connections of the MPOA

It would appear from the available literature that it is the lateral connections of the MPOA, primarily with the MFB, that are vital for the expression of maternal behaviour and other connections - with the ST for example - are relatively unimportant. Unfortunately, again, all work has been carried out on females.

In lactating female rats, bilateral parasagittal knife cuts transecting the dorsolateral neural connections of the MPOA severely disrupt maternal behaviour (Smotherman et al., 1977). In a later study, these lesions were found to preferentially abolish nest building and retrieving components of maternal behaviour, while crouching and nursing were unaffected. The authors suggest that these knife cuts interrupt the lateral connections of the MPOA with the MFB (Terkel et al., 1979). In a later study, lateral, anterior,

dorsal and posterior knife cuts were employed. Severing the lateral connections of the MPOA again severely disrupted maternal behaviour, while severing the dorsal or posterior connections of the MPOA produced either minor deficits or no deficits. Knife cuts anterior to the MPOA did also produce large deficits in maternal behaviour, but this was associated with hypoactivity and loss of body weight indicating a more generalised behavioural deficit (Numan & Callahan, 1980).

There is evidence that only certain components of maternal behaviour depend on the connections of the MPOA with the MFB (Miceli et al., 1983). In this study, virgin or post-parturient rats were subjected to near-lateral (NL) or far-lateral (FL) knife cuts. Only the NL knife cuts severed connections with the MFB. NL and FL knife cuts were equally effective in preventing the induction of maternal behaviour produced by the repeated exposure of virgin rats to foster pups. Both types of cuts also reduced nest building in virgins. In post-parturient rats, NL and FL cuts both abolished pup retrieval and reduced nursing behaviour, however only NL cuts disrupted lactation and nest building. The authors conclude that, while MPOA connections through the MFB are important for nest building and possibly lactation, other lateral connections must also be important for pup retrieval and nursing behaviour, though the identity of these connections is not known at present.

More recent work has concentrated on the neural target areas of the MFB fibres involved in maternal behaviour. Numan and Nagle (1983) implicated the substantia nigra (SN) in the central control system. Postpartum lactating rats that received large electrolytic lesions of the SN were found to have severely disrupted maternal behaviour. In order to test the hypothesis that the MPOA and SN interact in the control of maternal behaviour, postpartum lactating rats were subjected to asymmetrical lesions. Rats that received a unilateral knife cut severing the lateral connections of the MPOA and a

contralateral lesion of the SN showed larger deficits in maternal behaviour than either sham-operated females or females that had received a unilateral knife cut paired with an ipsilateral SN lesion.

A further study also implicates MPOA projections to the ventral tegmental area (VTA) (Numan & Smith, 1984). Bilateral electrolytic lesions of the VTA severely disrupted the maternal behaviour of postpartum rats. Also, lactating rats that received a unilateral knife cut severing the lateral connections of the MPOA paired with a contralateral lesion of the VTA showed more severe deficits in maternal behaviour than females that received one of the following treatments; (i) a unilateral knife cut severing the lateral connections of the MPOA coupled with an ipsilateral VTA lesion, (ii) a unilateral knife cut severing the lateral connections of the MPOA paired with a contralateral lesion of the medial hypothalamus posterior to the MPOA, or (iii) a unilateral knife cut severing the lateral connections of the LPOA paired with a contralateral VTA lesion. The oral components of maternal behaviour (retrieving and nest building) were particularly affected as a result of bilateral damage to the system extending from the MPOA to the VTA via the MFB.

2.2.4 Other neural areas

2.2.4.1 The olfactory system

Traditionally, maternal behaviour in mammals has been viewed as being under multisensory control, i.e. no one sensory modality is essential for the recognition of young and the performance of maternal activities by the mother. Research on rodents has indicated that olfactory, visual, auditory and other sensory modalities are all used by mothers in the efficient performance of their maternal activities (Numan, 1985). However, much

work has been carried out on the role of olfaction in maternal behaviour, mainly in rodents, and it is clear that this sensory pathway is of particular importance and that the multisensory theory may not be correct.

Some of the first evidence to indicate that this may not be the case comes from a study on the effects of olfactory bulbectomy on the maternal behaviour of lactating and virgin mice (Gandelman et al., 1971). Of 20 mice that underwent bulbectomy, 18 displayed no maternal behaviour, whereas 19 of 20 sham-operated controls showed maternal behaviour until weaning. Of the bulbectomised mice, 16 ate their young, with this cannibalism typically occurring within 12 hours of pup exposure, and 2 females just ignored their pups.

This bulbectomy-induced cannibalism has been partially explained by a later study (Fleming & Rosenblatt, 1974). This work suggests that the cannibalism observed in inexperienced, cycling virgin females after olfactory bulbectomy is not the result of anosmia but results from a disruption of non-olfactory influences of the bulb. In this study, bilateral bulbectomy resulted in a two-fold effect. Approximately 50% of the females cannibalized the young whilst, in the other half, there was a remarkable reduction in latencies to onset of maternal behaviour - most of these females retrieved and adopted a nursing posture over the foster young within 24 hours.

This difference between the two studies may be related to the fact that, in the first study, surgery was performed 1-11 days before pup exposure whilst, in the second study, females were allowed 1-3 weeks recovery. Interestingly, in the second study it was also found that two-stage bilateral olfactory bulbectomy resulted in females that exhibited short-latency maternal behaviour in the absence of cannibalism. It is possible that one-stage bulbectomy results in disruption of non-olfactory influences of the bulbs but, during 2-stage removal, some sort of reorganisation occurs following the first

stage which renders the second operation less effective (Fleming & Rosenblatt, 1974). This disruption may also be overcome with time, resulting in the reported facilitation of maternal behaviour following bilateral bulbectomy if the females are allowed time to recover from surgery before being exposed to pups.

From this evidence it would appear that pup odors delay the onset of maternal behaviour in virgins though it remains to be determined whether the observed reduced latency to onset of maternal behaviour is specifically related to the inability of virgins to smell pup odors. Furthermore, how pup exposure can override this presumed inhibitory effect remains to be elucidated. If pup odors do indeed inhibit the onset of maternal behaviour in virgins, the possibility arises that one of the ways in which the hormonal events of pregnancy may facilitate the normal onset of maternal behaviour at parturition is by altering the female's olfactory sensitivity (Numan, 1985). A report by Pietras and Moulton (1974) shows that hormones can influence odor detection in rats and, significantly, that odor detection performance is markedly depressed during pseudopregnancy. However, it remains to be shown whether the hormonal events that underlie the onset of maternal behaviour near the time of parturition actually depress olfactory sensitivity.

The accessory olfactory system also plays a role in maternal behaviour, as sectioning the vomeronasal nerves in virgin female rats also results in a more rapid onset of maternal behaviour as compared to controls (Fleming et al., 1979). However, although vomeronasal nerve cuts did result in more rapid maternal care, animals sustaining loss of both accessory and main olfactory function showed the shortest latencies of all, suggesting that the two systems work in parallel to tonically inhibit maternal behaviour in virgins. In summary, it would appear that olfaction inhibits maternal care in rats, and that olfactory bulbectomy results in shorter latencies to its onset. However,

there would also appear to be a non-olfactory influence of the bulbs that may result in cannibalism if surgery is performed immediately prior to exposure to pups, though the effects disappear if bulbectomy is carried out in two stages leaving time for neural reorganisation between operations.

2.2.4.2 The amygdalae

There has been little work on the role of the amygdalae in maternal behaviour although the work of Kling (1974) indicated a lack of general social interaction and maternal care in amygdalectomised female rhesus monkeys.

However, in a second study, lesions of the amygdalae resulted in a decrease in the latency of onset of maternal behaviour in nulliparous rats (Fleming et al., 1980). It was found that animals sustaining damage to the corticomedial amygdaloid nuclei became maternal more quickly than did animals sustaining basolateral amygdaloid damage. Also, in comparison to lesioned controls, animals with lesions of the amygdalae showed reduced 'fearfulness' on a number of fear-mediated tasks. These results are interpreted to mean that nulliparous females generally do not respond maternally to pups because these females are, in general, more neophobic than parturient females and they tend to find pups and their novel odors aversive. The authors conclude that this aspect of their behaviour is likely to be mediated by the amygdalae.

There would therefore appear to be a species difference in the effects of amygdalectomy on maternal behaviour; in primates this procedure results in social withdrawal and loss of maternal behaviour, and in rats maternal behaviour is stimulated. This may reflect the relative importance of the olfactory system in these two species as there is a large projection from the olfactory bulbs to the amygdalae (Scalia & Winans, 1975) and it has been

shown that bulbectomy also facilitates maternal behaviour in virgin female rats (see previous section).

2.2.4.3 The neocortex

Beach (1937) explored the role of neocortical mechanisms in the control of maternal behaviour of the female rat. Neocortical lesions of various sizes were produced in adult virgin female rats that were subsequently mated and their maternal behaviour was studied during the first 4 postpartum days. It was found that lesions involving less than 20% of the total neocortical surface produced only slight deficits in maternal behaviour though larger lesions produced more severe deficits, and when more than 40% of the cortex was destroyed, maternal behaviour was almost completely abolished. The degree of disruption of maternal behaviour was not related to the position of the lesion, but to its size. This is similar to the results reported by Beach on the effects of neocortical lesions on masculine sexual behaviour (see section 2.1.5.3).

More recent research on rhesus monkeys, however, has indicated that the anterior temporal cortex is particularly important in the control of maternal behaviour. The most striking effects of bilateral anterior temporal cortex removal on maternal behaviour were, (i) maternal placidity and lack of aggressive response, and (ii) loss of protective retrieval of the infants in threatening situations. However, passive acceptance of the infant was evident and nursing was generally tolerated (Bucher et al., 1970).

As mentioned in section 2.1.5.3, the temporal cortex is important in the integration of sensory inputs, especially visual cues, and one would therefore expect ablations within this area to have inhibitory effects on parental behaviour.

A major problem with interpreting these results (which also applies to the

effects of amygdalectomy) is that the monkeys in question exhibit severe social withdrawal and lack of contact with any individuals, so that the effects on maternal behaviour may only be an indication of a more generalised deficit in social interactions.

2.2.4.4 Other limbic structures

A number of studies have implicated other limbic structures in the control of maternal behaviour. Slotnick (1967) investigated the maternal performance of multiparous rats which had received lesions resulting in partial or total destruction of the cingulate cortex. These animals displayed the appropriate motor patterns involved in rat maternal behaviour, but they were displayed in a highly disorganised fashion. Within a few days however, this disorganisation disappeared in the home cage, but it reappeared during tests in a strange cage. Animals receiving only partial damage displayed less impairment and recovered more quickly.

Kimble, Rogers and Hendrickson (1967) examined the role of the hippocampus in maternal behaviour. As compared to controls which sustained only neocortical damage or were unoperated, the hippocampal-lesioned females showed high cannibalism rates, poor nest building, less time in a nursing posture and poor retrieval. However, they licked and manipulated their pups as much as controls did, suggesting that they were not avoiding them. Small lesions placed in the fimbria result in similar effects as seen following hippocampal or cingulate cortex lesions. Fimbria lesions produced deficits in nest building and pup retrieval in particular, multiple nests were constructed and the pups retrieved to different nests (Terlecki & Sainsbury, 1978). Similarly, rats with lesions in the septum also display poorly integrated retrieval and virtually no nest building or nursing postures (Slotnick, 1969).

These results all indicate a role for limbic structures in the temporal and spacial patterning of all aspects of maternal behaviour, rather than in the control of the individual components of the complex.

2.2.5 Neurotransmitters implicated in the control of parental behaviour

Monoamines, particularly noradrenaline, have been implicated in the initiation of maternal behaviour (Moltz et al., 1969; Rosenberg et al., 1977) and brainstem lesions of the dorsal bundle, depleting noradrenaline in the cortex and hippocampus, result in deficits in the onset of maternal behaviour in primiparous rats. Similarly, fornix-bundle transections, depleting only hippocampal noradrenaline, are associated with an absence of pup care though hypothalamic noradrenaline levels are not significantly affected by these manipulations (Steele et al., 1979).

More recently, researchers have investigated levels of neurotransmitters in the cerebrospinal fluid (CSF) during parturition and maternal behaviour in sheep. Levels of acetylcholinesterase are significantly elevated during labour, parturition, lamb separation and suckling. CSF levels of oxytocin are also raised during labour, parturition, vaginocervical stimulation and suckling (Kendrick et al., 1986). In a later study (Kendrick et al., 1987), intracerebroventricular infusions of oxytocin were found to stimulate maternal behaviour in the sheep.

It would appear that the substantia nigra and olfactory bulbs play a role in this increase in intracerebral oxytocin, as levels within these specific neural loci have been found to increase during parturition and suckling (Kendrick et al., 1988a), as have levels of dopamine in the same areas.

Oxytocin levels within the olfactory bulbs have also been found to increase on vaginocervical stimulation and levels of γ - aminobutyric acid (GABA), aspartate and glutamate within the olfactory bulbs increase in the same way, indicating that vaginocervical stimulation produces significant changes in neurochemical release in the olfactory bulbs of sheep (Kendrick et al., 1988b). The authors suggest that such changes may be involved in the induction of maternal behaviour or in the olfactory recognition of offspring.

CHAPTER 3

MATERIALS AND METHODS

3.1 Introduction

This chapter contains details of all the materials and methods used in the course of this study. Each experimental chapter will refer the reader to the relevant section within this chapter, and will give details of experimental protocol.

3.2 The common marmoset (*Callithrix jacchus*)

There are very few primate species on which a study of this nature can be undertaken. Primates are, in general, difficult and expensive to keep and to breed in captivity and have a long developmental period.

The common marmoset does not present these problems; it has been bred in captivity for over 50 years (Lunn, 1978) and its behavioural repertoire has been extensively studied in a laboratory environment. In captivity, marmosets breed all year round with an inter-birth interval of approximately 156 days (Stevenson & Poole, 1976) and reach sexual maturity at approximately 18 months (Abbott & Hearn, 1978). This high reproductive rate compared with many other primate species is one of the major advantages of studying the marmoset. Marmosets are small, with an average body weight of approximately 350g which makes them relatively easy to handle in a laboratory situation. Another important factor is that female marmosets continue to show sexual proceptivity and receptivity post-ovariectomy (Kendrick & Dixon, 1984b) - see later for details of these behaviours - which makes hormonal treatments with progesterone and oestradiol prior to sex tests unnecessary. There has been extensive work carried out on the hormonal control of sexual behaviour in this primate

species (Kendrick & Dixon, 1983; 1984b; 1985a; 1985b), and finally there is a stereotaxic brain atlas available which forms the basis for manipulations of the hypothalamus in marmosets (Stephan et al., 1980).

The common marmoset is a New World primate that belongs to the family Callithrichidae. Callithricids are distinguished from all other anthropoids by their small size, by having claws instead of fingernails, by their dental formulae, and by the fact that about 80% of the litters contain twins (Goldizen, 1986). The Callithrichidae include two genera of tamarins: *Saguinus* (10 species) and *Leontopithecus* (1 species); and two genera of marmosets: *Callithrix* (3 species including *jacchus*) and *Cebuella* (1 species) (Hershkovitz, 1977).

Callithrix jacchus inhabits small remnants of rainforest along the Atlantic coast of Brasil and is now rare in the wild due to habitat destruction caused by an increasing human population. Marmosets are diurnal, arboreal and eat tree exudates (gum and sap), insects and fruit, having distinct morphological specializations for their feeding habits. Thus, marmosets have lower incisors as long as their canines, which they use to gnaw holes in trees, causing exudate to flow. However, the common marmoset has not been well studied in the wild (only 4 detailed field studies to date) and the importance of exudate in their diet is not known, though anecdotal evidence suggests that it is an important part of their nutrition.

Until recently it was thought that the common marmoset was monogamous and lived in small family groups consisting of an adult breeding pair together with their offspring. This was mainly inferred from laboratory studies in which new adults introduced into a stable group were not tolerated and driven out, although young born to the breeding pair may remain in that group for many years without aggression (Eppler, 1970a). In family groups in captivity, daughters that have reached sexual maturity are inhibited from

reproduction whilst they remain in the group, but once removed and paired with a male they quickly become pregnant (Abbott et al., 1981). However, more recently there has been evidence that wild groups of marmosets contain several adult males and females in groups that vary in size from 3-13 and the turnover in group membership can be high (Hubrecht, 1984; Rylands, 1986; Scanlon et al., in press). In a population of six groups of individually marked tamarins (also thought to be monogamous at one time), which were monitored for a 4 year period, a high degree of variability in the groups' mating systems were seen, including some evidence of polyandry (Terborgh & Goldizen, 1985). It is now thought that there is variability within species with respect to their group composition, but further long-term studies are required before a categorical answer on the normal group composition of *Callithrix jacchus* in the wild can be given.

As previously mentioned, the interbirth interval of marmosets in captivity is approximately 156 days, with a gestation period of 148 days. Females show a post-partum LH surge 10 - 18 days after birth and generally conceive during this cycle (Dixon & Lunn, 1987). In the wild there is some evidence for seasonality of breeding, with births occurring in two peaks; one at the beginning of the dry season, i.e. late October to November and the other near the start of the wet season, i.e. late March to April. The inter-birth interval in the wild therefore lies between 5 and 7 months (Hubrecht, 1984). The mean cycle length of female marmosets in captivity is 27.7 days (range 24 - 30 days), the follicular phase lasting on average 8.7 days (range 4 - 12 days) and the luteal phase 19.0 days (range 14 - 24 days). Females show sexual proceptivity and receptivity throughout the cycle, with a peak in proceptivity occurring around the time of ovulation (Kendrick & Dixon, 1983).

The high rate of reproduction seen in the common marmoset is possible due

to the substantial help given by the father and older siblings. This high level of paternal care is seen throughout the *Callithrichidae* species, and also in Owl monkeys (Dixon & Fleming, 1981) and Siamangs (Alberts, 1987). Male involvement in the care of marmoset young includes transportation and grooming of the twins for the first few weeks; later on the father helps to encourage the independence of the youngsters, shares his food with them, plays with them, and protects and defends them against intruders - see later in this chapter for a description of the paternal behaviour scored in this study. However, there is much individual variation regarding the timing of onset of this behaviour post-partum and its intensity, and there may even be variation within individuals between successive pregnancies (Goldizen, 1985).

The behavioural repertoire of the common marmoset in captivity has been described in detail elsewhere: general social behaviour (Epple, 1975a; Stevenson & Poole, 1976), social interactions among individuals (Box, 1984; Evans & Poole, 1984), parental behaviour (Ingram, 1977), aggression (Lipp, 1978), scent marking (Epple, 1970b; Sutcliffe & Poole, 1978) and sexual behaviour (Kendrick & Dixon, 1984a). The specific behaviours scored in the course of this study are described in detail later in this chapter.

3.3 Colony management

Stock animals and the breeding colony were housed in family or peer groups in cages 51 x 51 x 75 cm. The breeding groups were allowed access once a week to an exercise cage measuring 180 x 60 x 180 cm. The group size and makeup varied but, on average, about 6 animals from a family were kept together. Adolescent animals were removed from family

groups and set up in breeding pairs or peer groups when the groups became too large, or fighting occurred.

All animals in the colony were fed once daily on a mixture of Mazuri monkey chow (SDS Diets, Essex), a selection of fresh fruit (apples, bananas, carrots, grapes, tomatoes, pears, oranges), peanuts, sunflower seeds, raisins and three times a week this diet was supplemented with high protein porridge (Casilan, Build-up, Cytaccon). Water was available *ad libitum*.

Natural light was supplemented by artificial lighting from 0600h to 1800h in the colony and behaviour rooms. The temperature was maintained at approximately 70 - 75°F and the humidity between 45 and 50%. Further details of the colony management are described elsewhere (Kendrick & Dixson, 1984a).

3.4 Animal selection

Fig. 3.1 shows details of all the animals used in the course of this study, their date of birth, age when used, the experiment that they were involved in, and, in the case of the females, the date of ovariectomy. The criteria used to select animals for either the pair test experiments or the permanent group situation are outlined below.

3.4.1 Pair test individuals

Selection of individuals from the colony for the pair testing experiments was based on a number of criteria. The age of the animals was 6.1 years \pm 0.6 (mean \pm sem). Common marmosets reach sexual maturity at approximately 18 months (Abbott & Hearn, 1978). The females were ovariectomised prior to the commencement of the experiments - see Fig. 3.1.

Individuals of average age and body weight (mean 344.4g +- sem 13.2) were singly housed and pair tested for 1-2 weeks with the same partner. If, after this time, the pair were copulating regularly on each test, they were included in the experimental group. If however, regular copulation was not achieved, the partners were changed, or the individuals discarded from the study.

3.4.2 Parental behaviour groups

The selection procedure for these individuals was based on different criteria to that for pair test animals. All the females were parous and had successfully raised at least one set of offspring with the present male partner. The ages of the individuals were comparable with those in the pair tests (mean 4.8 years +- sem 0.4). Pairs were chosen 2-3 weeks before the expected date of delivery - calculated from interbirth intervals and uterine palpations - and placed in the video room. Any previous offspring were separated from their parents for the duration of the study.

No.	D.O.B.	Age	D.O.O	Exp	No.	D.O.B	Age	D.O.O	Exp
27R	08-06-75	11	06-10-76	1+2	275R	14-09-82	5	02-05-85	1+2
115R	02-07-77	9	09-06-82	1+2	279R	25-09-82	5	-----	3
184R	10-05-80	6	29-04-84	1+2	289R	19-04-83	4	-----	3
185R	10-05-80	6	20-01-84	1	293R	25-05-83	4	07-05-87	2
226R	10-04-81	5	25-04-85	1	316R	19-03-84	3	02-04-86	2
237R	26-07-81	6	01-05-85	1+2	317R	29-03-84	3	-----	3
242R	21-09-81	6	22-04-85	1	321R	18-05-84	3	-----	3+4
247R	12-03-82	5	-----	3	332R	02-09-84	3	-----	3+4
271R	30-08-82	5	-----	3	338R	18-12-84	4	09-12-87	2
272R	03-09-82	5	18-04-85	1+2					

Fig. 3.1a Details of the females used in this study.

No.	D.O.B.	Age	Exp	No.	D.O.B.	Age	Exp
44BK	18-07-75	11	1	262BK	03-05-82	5	2
141BK	12-07-78	8	1	268BK	20-05-82	4	1
152BK	24-10-78	8	1	272BK	03-07-82	5	2
170BK	07-06-79	8	1+2	274BK	30-08-82	5	3
181BK	03-10-79	7	1	276BK	14-09-82	4	1
183BK	18-10-79	7	1	280BK	27-06-82	5	3
186BK	03-12-79	7	1	282BK	01-11-82	5	2
189BK	07-12-79	8	3	283BK	06-01-83	3	1
190BK	07-12-79	7	1	287BK	30-03-83	3	1
195BK	16-03-80	6	1	290BK	19-05-83	3	1
204BK	26-04-80	6	1	291BK	21-05-83	4	2
209BK	05-06-80	6	1	302BK	30-07-83	4	3+4
220BK	03-10-80	6	1	303BK	14-08-83	4	3
228BK	29-12-80	6	1	313BK	07-12-83	3	1
234BK	10-04-81	6	3	315BK	29-12-83	3	1
246BK	09-07-81	5	1	318BK	06-03-84	3	3+4
248BK	17-08-81	7	1+2	328BK	15-05-84	2	1
251BK	05-10-81	5	1	331BK	19-05-84	3	2
254BK	15-03-82	4	1	339BK	02-10-84	2	1
255BK	12-04-82	4	1	340BK	18-12-84	3	2
260BK	24-04-82	4	1	349BK	26-05-85	2	1+2
261BK	25-04-82	5	2				

Fig. 3.1b Details of the males used in this study.

No. = Animal number, R = female, BK = male
D.O.B. = date of birth
Age = age at time of experimentation, in years
Exp. = experiment (s) in which the individual was used - see chapter titles for description.
D.O.O. = date of ovariectomy (---- = intact)

3.5 Pair test procedure

Males and females used in the pair test experiments were singly housed, in cages of dimensions 62 x 51 x 76 cm, containing a shelf at the back and a hard-wood perch at the front. The bases of the cages were covered in woodshavings. The cages were arranged in blocks of 4, with males in the left hand cages and their respective female partners in the adjacent right hand cages. All cages had external removable nest boxes which could be fitted to any cage.

All pair tests were conducted in the males' home cages with observations being made from behind a one-way mirror - see Fig. 3.2. Water bottles were removed from the males' cages and a tray of fresh shavings was placed in the base of the cage before the commencement of each test. This ensured that the animals did not eat or drink during the test, which could have acted as a distraction.

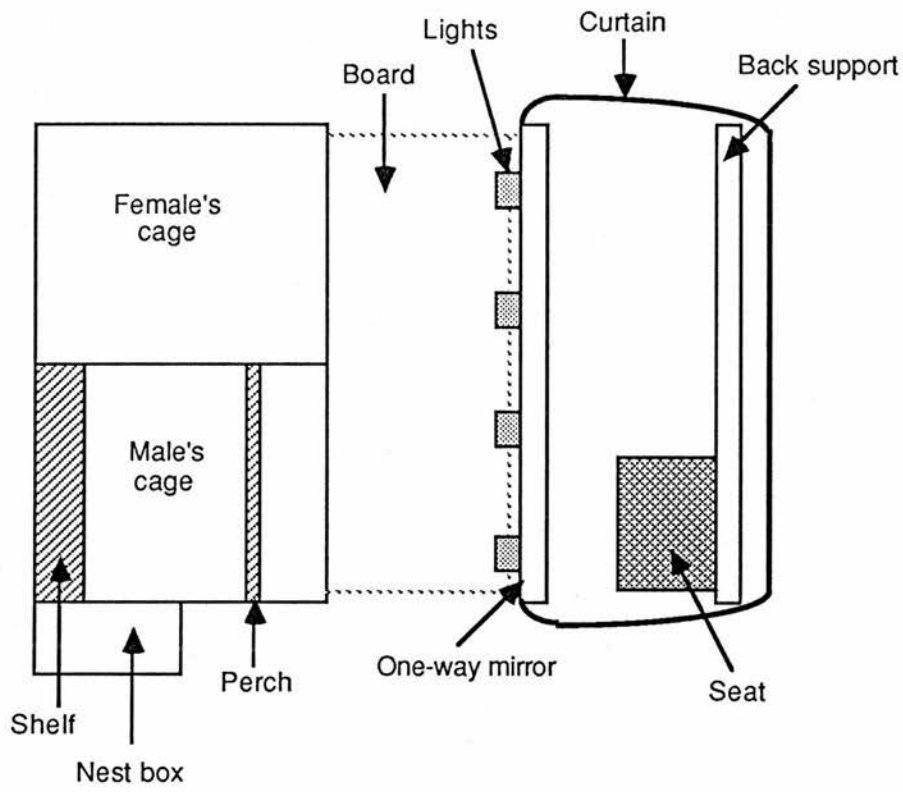


Fig. 3.2 Plan view of pair test cages and observation procedure - see text for details.

At the commencement of the test, the male's nest box was removed and the female's one (containing the female) was substituted. A sheet of hardboard was then inserted between the upper and lower cages to prevent the distraction of reflections of other animals showing in the mirror. The male's cage was then illuminated with 4 60W bulbs to ensure clear visibility for the observer during the test. These lights were connected to the front of the

screen holding the mirror which was placed 1-2 feet in front of the cage. A second screen, which had a seat attached, was placed behind the first and a black curtain was draped over the entire set up. This ensured that the observer could clearly see the animals and that the animals were unable to see the observer.

Treatment _____		Date / /					Test no. _____				
Anticipatory erection? Yes / No											
	MALE					FEMALE					
Allogrooming											
Grooming invitation											
A.G. sniffing / licking											
Scent marking											
Genital present											
Aggression											
Proceptive bout											
Total tongue flicks											
	Mount refusals										
	Mount terminations										
	1	2	3	4	5	6	7	8	9	10	
No. mounts											
Erection?											
Thrusting?											
Female initiates?											
	Time / clock					Time / actual					
Mount latency											
Intromission latency											
Ejaculation latency											
PEI											
IEI											

Fig. 3.3 Layout of the score sheets used during pair test experiments.

AG = anogenital PEI = post-ejaculatory interval
 IEI = inter-ejaculatory interval

The test commenced on entry of the female into the male's cage from the nest box. The entrance to the nest box was then closed for the duration of the test to ensure that the animals were in view at all times. All individuals used in the pair test situation were extensively habituated to the procedure before commencement of the experiments and showed no adverse reactions to the method, copulating on the majority of tests.

Behaviours were scored using a series of stop watches and hand counters attached to a clip board and recorded on sheets, an example of which is shown in Fig. 3.3.

3.6 Video recording procedure

The pairs of marmosets used in the permanent parental group situation were observed via a time-lapse video recording system. This system was set up in a specialized room with no natural lighting. Artificial lighting, in the form of a fluorescent strip suspended above the cage, was automatically switched on at 0600h (GMT) and off at 1800h every day. The humidity was maintained at approximately 45% and the temperature between 70 and 75°F.

The groups were housed in a weld-mesh cage with a perspex front, measuring 60 x 60 x 53 cm. - see Fig. 3.4. The cage contained a shelf at the back and a hard-wood perch at the front. There was a mesh base with a tray of shavings underneath. Water was available from a water bottle attached to the side of the cage.

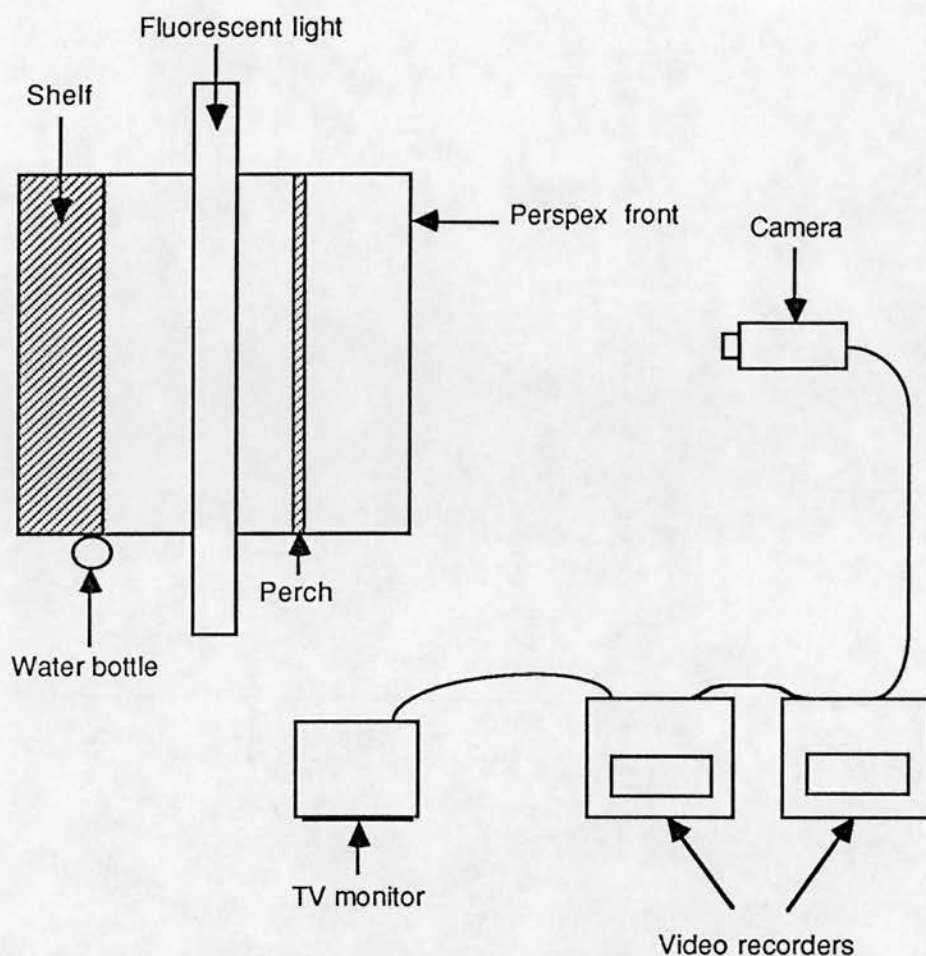


Fig. 3.4 Plan view of video cage and monitoring procedure - see text for details.

A Panasonic TV camera (model WV-1550/B) was situated in front of the cage and linked to two Panasonic video recorders (monochrome model NV-8050) located in a separate room. These operated throughout the light period at 4X normal speed and also recorded the date and time continuously. It was found that the resolution on tapes played back at this speed was clear enough to score all aspects of the behaviours of interest accurately. Fuji E-180 Super HG VHS video cassettes were used and these were played back via a Panasonic remote control facility (model NV-A810) and an Hitachi high resolution monitor (model VM 129E/K).

The pairs of marmosets were placed in this video room 2-3 weeks pre-partum (calculated from inter-birth intervals and uterine palpations) for a

period of habituation. There were no signs of distress from any of the pairs during habituation to this novel environment.

3.7 Olfactory discrimination tests

Olfactory discrimination tests were carried out in the males' home cages using the same apparatus as for a pair test, though no female entered the cage.

Two identical dishes of food were used in each test but one was sprinkled with 0.2ml n-Caproic acid (Sigma, Poole, Dorset, U.K.) - a noxious highly-volatile fatty acid. This had previously been seen to cause an avoidance reaction in response to olfactory investigation in normal marmosets when presented on a cotton bud. Each food dish was then covered with clear plastic that had been repeatedly punctured with a needle to allow the animals to smell the contents. This plastic was firmly attached to the dishes to prevent the animals from removing it and reaching the food.

For each test, one tray of treated food and one of normal food was presented to each male in the base of his cage. The dishes were placed one on the left and one on the right, and the position of the treated and normal food was randomized between tests. During placement of the dishes in the cage, the male was locked in his nest box - the tests commenced on his re-entry into the cage.

Approaches to each dish were scored, as well as the number of times that the male attempted to grab a piece of food from either dish ('touch'). The tests lasted for 10 minutes, or until the male had ripped the plastic covering on either dish. At no time during any of the tests did a male remove or taste a piece of food from either of the dishes in his cage.

3.8 Behaviours scored

Many aspects of the behavioural repertoire of the common marmoset have been described in detail elsewhere; sexual behaviour (Stevenson & Poole, 1976; Kendrick & Dixson, 1984a), scent marking (Epple, 1970b; Sutcliffe & Poole, 1978), genital presents (Stevenson & Poole, 1976), agonistic behaviours (Lipp, 1978) and parental behaviour (Ingram, 1977; Vogt et al, 1978). The following account gives precise details of the definitions of each behaviour used in this study.

3.8.1 Sexual behaviour

Male

- i) Anticipatory erection. The presence or absence of an erection prior to entry of the female to the males' home cage was noted during pair tests. Males rapidly habituate to the test procedure and achieve an erection during the movement of the cages and lights.
- ii) Number of mounts. A mount was scored when the male achieved a position dorsal to the female whilst touching her flanks or hips and from which intromission would be possible.
- iii) Number of intromissions. Common marmosets normally achieve a single intromission on each mount after a series of rapid pelvic thrusts. Intromission is characterized by an alteration in this thrusting pattern in that the thrusts are deeper and slower. The females' behaviour also changes dramatically on intromission - she immediately begins to struggle and turn her head to bite at the male. This struggling behaviour was not scored as a mount termination as it occurs on 80% of ejaculatory mounts and is therefore thought to be a normal behavioural sequel to ejaculation rather than a reflection of the female's unwillingness to mate (Kendrick & Dixson, 1984a).

- iv) Number of ejaculations. Males will invariably ejaculate on intromission after several deep pelvic thrusts. Ejaculation is characterised by abrupt cessation of this thrusting activity and termination of the mount by the male or the female. The males will invariably groom their anogenital region after ejaculation and there is a pulsating movement of the penis which has been previously found to occur after 100% of ejaculations as evidenced by vaginal lavages (Kendrick & Dixson, 1984a). In the majority of cases the female will scent mark with her anogenital glands immediately after an ejaculation.
- v) Mount latency. This latency is the time, measured to the nearest second, from the entry of the female to the male's home cage, to the first mount or attempted mount.
- vi) Intromission latency. The latency, measured to the nearest second, from entry of the female to the male's home cage to the first intromission. In the case of the parental behaviour study, intromission latency was calculated as the time from the first mount during a bout of sexual activity to intromission.
- vii) Ejaculation latency. The latency, to the nearest second, from the first intromission to the first ejaculation. As, in most cases, only one ejaculation occurred per test, and post-ejaculatory mounts were seldom seen, measurements of the post-ejaculatory interval or inter-ejaculatory interval were therefore not considered in any of the studies.
- viii) Male precopulatory bouts. Male marmosets show certain behaviours before mounting which bear resemblance to female proceptive behaviour and appear to indicate sexual interest. As in the female, rapid tongue flicking movements are seen in response to eye contact with the female in most cases. The number of these tongue flicking bouts was scored in each test.
- ix) Total male tongue flicks. The total number of tongue flicks seen during a test was counted. Rhythmic mouth movements are also seen but, to achieve

a score, the tongue had to be seen protruding from the mouth.

x) Characteristics of each mount. For each individual mount, it was determined whether an erection was present, whether pelvic thrusting occurred, and whether the mount was initiated by the male or the female.

xiv) Hip touches. In some post-lesion tests a new type of behaviour was seen. In this case, the male would approach the female's hind quarters, grip her hips or back and appear ready to mount. However, the male would then appear to lose interest and a mount would not occur.

Female

i) Female proceptive bouts. Female marmosets exhibit 'freezing' behaviour during which the animal crouches, draws back its ear tufts and stares at the male. During this behaviour, tongue flicking may or may not be seen. A proceptive bout was defined as a period of 'immobility' by the female with the associated crouching, flattening of the ear tufts and staring at the male. A bout was deemed over when the female moved from the crouching immobile position. A bout was scored whether or not tongue flicking occurred though, in some cases, the presence or absence of this behaviour was noted (see Chapter 4).

ii) Mount refusals. A mount refusal was scored if the female moved away or showed aggression towards the male (vocal or physical) on approach with the intention of mounting i.e. approach towards the female's hind quarters and hand contact with the female's legs or hips. In the case of a mount refusal, the male's behaviour was scored as a mount attempt.

iii) Mount termination. This involved movement of the female away from the male during a mount, and / or aggression, leading to termination before intromission. The struggling behaviour previously described (see male - iii) that occurs on intromission was not scored as a mount termination.

3.8.2 Social behaviour

- i) Grooming. Two types of grooming were scored during this study. Allogrooming occurred between males and females and also between parents and offspring (during the parental behaviour study). This consisted of manipulation of the fur with the hands and / or mouth for a period of 4 seconds or more. This was scored as one bout, and had to be separated from a second bout by 4 seconds or more to achieve a score. The second type of grooming, anogenital grooming, was scored separately from allogrooming in the parental behaviour studies described in Chapters 6 and 7.
- ii) Grooming invitations. This behaviour is characterised by an individual stretching out in front of, or presenting part of its body to another animal in an attempt to initiate grooming behaviour. Each stretch or presentation was scored as an individual grooming invitation.
- iii) Scent marking. The incidence of this behaviour was scored only for the males and was characterised by the rubbing of the anogenital region (with or without urination) or the thoracic region against the cage or the perches. Each bout of rubbing was scored as one scent mark.
- iv) Genital present. The occurrence of this behaviour was scored only for the males and is characterised by piloerection and presentation of the genitalia by flexion of the tail. The present was directed towards other individuals or at random.
- v) Aggression. During the pair tests, any physical aggression (consisting of lunges, hits or bites) or vocal aggression between the two individuals was scored and the instigator of the activity was noted.

3.8.3 Agonistic behaviour

In the case of the tests where a strange male was introduced into the

experimental male's home cage in place of a female, aggressive and submissive behaviours were scored in more detail.

- i) Attack. This behaviour is characterised by physical contact between the two individuals by either hitting or biting.
- ii) Lunge. In this case, the aggressor moves rapidly towards his opponent though body contact is not made.
- iii) Chase. A chase involves the aggressor pursuing the opponent round the cage without any physical contact.
- iv) Vocal threat. This is characterised by a 'chattering' vocalization repeated frequently.
- v) Arch ruffle. This behaviour is recognisable by piloerection normally accompanied by 'stiff-legged' walking up and down a perch.
- vi) Crouch. This is a submissive behaviour in which the subordinate animal normally attempts to occupy a position in the cage below the level of the dominant animal, and crouches.
- vii) Flee. The subordinate animal escapes from the aggressor in response to physical aggression or threat behaviour.
- viii) Squeal. A further example of submissive behaviour is that of squealing - a high-pitched vocalization which normally occurs in concert with fleeing or grimacing.
- ix) Grimace. This behaviour is characterised by the withdrawal of the lips from the teeth and is another example of submissive behaviour.
- x) Tongue flicking. This has been described previously as a proceptive behaviour, but in the case of a confrontation between two males, this same behaviour would appear to be a submissive gesture.

3.8.4 Parental behaviour

- i) Carrying time. This behaviour was scored by calculating the total time

during which each parent carried one or both infants. In the case of the female, the length of time that the infants spent in the suckling position was also noted. Due to lack of clarity on the video monitor, it was impossible to determine whether the infants were actually suckling, so the times quoted will probably be an over-estimate of actual suckling as the infants would also sleep on their mother's ventral surface. The time that the infants spent loose in the cage without contact with either parent was also scored, as 'time off'.

ii) Transfers. Movement of the infants between the two parents was scored, and the instigator of the transfer was also determined. In the case of the parents, this would involve the removal of an infant from one parent by the other or one parent handing an infant to the other. Infants were also seen to initiate transfers both between parents, and between parents and the cage.

iii) Dislodge. Dislodge behaviour is seen for both parents and is characterised by rolling, rubbing and biting in an effort to remove the infants by the carrier. This behaviour pattern would either result in the removal of the infants to the cage, or the arrival of the second parent and subsequent transfer to that individual.

iv) Re-establishment of contact. Once the infants were loose on the cage during 'time off', it was determined how re-establishment of contact was made. Either the parents would retrieve the infants, or the infants would attempt to climb back on to the adults. The adult involved would either accept or reject this re-establishment; both acceptances and refusals by the parents were scored.

3.8.5 Other behaviour patterns scored

i) Locomotion. To score locomotor activity, the cage was divided into 8 equally sized quadrants and movement of the animal between quadrants was scored.

- ii) Eating. A bout of eating behaviour was scored no matter how long it lasted though each successive bout was separated by at least 4 seconds.
- iii) Drinking. A bout of drinking from the water bottle was scored no matter how long it lasted though each successive bout was separated by at least 4 seconds.

3.9 Surgical procedures

3.9.1 Ovariectomy

The females were starved overnight and then anaesthetised with ketamine hydrochloride (Vetalar, Parke-Davis, Gwent, U.K. - 0.05ml i.m.) and Saffan (Glaxovet, Middlesex, U.K. - 0.2ml i.m.). The ventral surface of the animal was shaved and swabbed with iodine followed by 70% alcohol. A midline incision was made in the skin and abdominal wall. Muscle and fat was cleared using a blunt dissection technique until the uterus was exposed. This was lifted clear of the body to expose the ovaries. All tissue was kept moist with sterile physiological saline throughout the course of the operation. The ovarian blood vessels and Fallopian tubes were ligated with catgut (Ethicon, Edinburgh, U.K.) and the ovaries removed. The peritoneum and abdominal wall were sutured with catgut, and the skin with silk suture (Mersilk, Ethicon, Edinburgh, U.K.). The females were all treated with a broad-spectrum antibiotic post-operatively (Ethicilin, Intervet, U.K. - 0.2ml i.m.). The females were then placed in an incubator until consciousness was regained. Post-operative recovery was uneventful in all cases.

3.9.2 Subcutaneous implantation of oestradiol capsules

Implants were constructed from 29mm lengths of silastic tubing with an

internal diameter of 0.058" (Medical-Grade Tubing, Dow Corning, Michigan, USA). One end was sealed with Medical adhesive, Silicone Type A (Dow Corning, Michigan, USA) and the implants dried over night at 60°C. The implants were then packed with 17 β -oestradiol (Sigma, Poole, Dorset, UK) and the second end sealed with adhesive. This was again allowed to harden over night at 60°C. Prior to subcutaneous implantation, the silastic implants were pre-incubated over night in sterile physiological saline at 37°C to absorb the initial surge of oestradiol released from the implants at this temperature.

All females receiving oestrogen implants were starved over night and anaesthetized with the short acting anaesthetic, methoxitane sodium (Brietal Sodium, Eli Lilly & Co., UK - 0.4ml of 1% solution i.v.). This resulted in anaesthesia for a period of 8 - 12 mins. An area of the lower back was shaved and swabbed with iodine followed by 70% alcohol. A small incision was made and two oestradiol implants inserted subcutaneously into each animal. A single suture using 3/0 chromic suture (Ethicon, Edinburgh, UK) was sufficient in most cases.

This administration of oestradiol has been previously shown to produce plasma levels of oestradiol of 940 \pm 150 pg/ml (mean \pm s.e.m.) in ovariectomized female common marmosets (Kendrick & Dixon, 1985a). These plasma levels are comparable with periovulatory values and have been shown to produce measurable increases in proceptive behaviour in female marmosets (Kendrick & Dixon, 1985a).

3.9.3 Thermal MPOA-AH lesions

Thermal and sham lesions were performed under ketamine hydrochloride (Vetalar, Parke-Davis, Gwent, UK - 0.05ml i.m.) and Saffan (Glaxovet, Middlesex, UK - 0.2ml i.m. with repeated doses of 0.2ml as required)

anaesthesia. Males were placed in a stereotaxic frame (Kopf Instruments, Tujunga, USA) using ear and eye bars designed for use with the squirrel monkey. The scalp was shaved and the head washed with iodine followed by 70% alcohol and distilled water. A midline incision was made and the periosteum scraped away with a scalpel blade. A midline hole was then drilled using a dental drill and the dura punctured with a hypodermic needle to allow passage of the lesioning electrode. Thermal lesions were made using a Kopf RFG-4 lesion generator and a K1388Z probe. A temperature of 55°C was produced for 15 seconds bilaterally at two depths, as described below. In the case of the sham lesions, the electrode was lowered but no current passed. The hole was then plugged with bone wax (Ethicon, Edinburgh, UK) and the scalp sutured using 3/0 chromic suture (Ethicon, Edinburgh, UK). All males were then treated with an antibiotic (Ethicilin, Intervet, U.K. - 0.2ml i.m.) and placed in an incubator until consciousness was regained.

The stereotaxic coordinates used were those from the atlas of Stephan et al (1980). AP coordinates are measured from a zero at the inter-aural plane, verticals from a zero at the base of the brain, and laterals from a zero at the midline. In the case of the AP coordinate, a correction factor of +1mm was required. This apparent discrepancy may have been due to the difference in body weights of the animals used in the atlas (mean 281g) and those used in this study (mean 344.4g). The coordinates used were AP +9.7mm, V +7.5mm + +6.8mm, L \pm 0.5mm (7 males), AP +10.0mm, V +7.5mm + +8.5mm, L \pm 0.5mm (1 male) and AP +10.3mm, V +8.0mm + +9.0mm, L \pm 0.5mm (5 males) for the lesioned males and AP +10.7mm, V +8.5mm, L \pm 0.5mm (4 males) or AP +10.3mm, V +10.5mm, L \pm 0.5mm (3 males) for the sham-operated individuals. The co-ordinates given refer to the atlas measurements, a correction factor of +1mm was then added to the AP

values. Details of the co-ordinates used in each phase of the study are given in the relevant experimental chapters.

Recovery progressed normally in all but one of the males that received thermal hypothalamic lesions though there was a tendency towards hyperphagy in the first 2-3 weeks post-operatively (average weight gain 10.8%). However, after this initial hyperphagic phase, the males were able to maintain their body weights at this new set point. In the case of the last male, feeding behaviour stopped for 3-4 weeks post-operatively and hand feeding was necessary 4 times per day to maintain body weight. After this period, the animal resumed eating and maintained a body weight of 95% of its pre-operative weight for the duration of the study.

3.9.4 Olfactory bulb sections

Olfactory bulb sections were performed under ketamine hydrochloride (Vetalar, Parke-Davis, Gwent, UK - 0.05ml i.m.) and Saffan (Glaxovet, Middlesex, UK - 0.2ml i.m. with repeated doses of 0.2ml as required) anaesthesia. Males were placed in a stereotaxic frame (Kopf Instruments, Tujunga, USA) using ear and eye bars designed for use with the squirrel monkey. The scalp was shaved and the head washed with iodine followed by 70% alcohol and distilled water. A midline incision was made and the periosteum scraped away with a scapel blade. A midline hole was then drilled using a dental drill at an atlas AP co-ordinate of +20.8. This AP position coincided with the anterior pole of the cerebral hemispheres. The bone anterior to this point was removed with the drill and forceps until the olfactory bulbs were visible, laying in a canal of bone.

Once a length of the bulbs had been uncovered, a hooked probe was used to pass a loop of suture (4/0 chromic, Ethicon, Edinburgh, UK) underneath. The two pieces of suture were then tied, one at the anterior extent of the

olfactory bulbs, and one at the junction between the olfactory bulbs and the anterior cerebral hemispheres. Care was taken to ensure that all neural tissue and blood vessels were ligated before the olfactory bulbs were sectioned between the sutures. The cut ends of the bulbs were pulled apart to ensure that all the neural tissue had been sectioned, and a plug of bone wax (Ethicon, Edinburgh, UK) was then placed between the cut ends to prevent neural regeneration. The hole was then plugged with bone wax and the scalp sutured using 3/0 chromic suture. The males were treated with a broad-spectrum antibiotic (Ethicilin, Intervet, U.K. - 0.2ml i.m.) and placed in an incubator until consciousness was regained.

3.9.5 Chronic intracerebral cannulations

Bilateral intracerebral cannulae were implanted under ketamine hydrochloride (Vetalar, Parke-Davis, Gwent, UK - 0.05ml i.m.) and Saffan (Glaxovet, Middlesex, UK - 0.2ml i.m. with repeated doses of 0.2ml as required) anaesthesia. Males were placed in a stereotaxic frame (Kopf Instruments, Tazunga, USA) using ear and eye bars designed for use with squirrel monkeys. The scalp was shaved and the head washed with iodine followed by 70% alcohol and distilled water. A midline incision was made and the periosteum scraped away with a scapel blade. The skull was then dried very thoroughly and was kept completely dry for the remainder of the surgery. The coordinates for the placement of the two cannulae were derived from the atlas of Stephan et al (1980) though, again, a 1mm correction factor was added to the AP coordinate. The atlas coordinates used were AP +9.7 and a final L of +1.0. It was necessary to implant at least one of the cannulae in each male at an angle towards midline to allow space for the cannulae caps. The guide cannulae implanted (Clark Electromedical Instruments, Reading, Berks. U.K.) were 8mm in length.

The protocol for the correct placement of the cannulae is outlined below:

1. Skull measurements were taken at the desired final lateral position i.e. ± 1 mm (Skull 1) and the lateral position at which the cannula was to be inserted (Skull 2).
2. It was then possible to calculate the distance from the skull (Skull 1) to the desired depth of the tip of the inner cannula (length B) by adding the baseplate V reading to the desired V coordinate. This sum was then subtracted from the Skull 1 reading to give the length B.
3. If the cannula was to be inserted vertically, the surgery could continue with no further calculations. However, if a lateral angle was necessary, the size of this angle was calculated as follows.
4. To calculate angle x:

$$\tan x = \frac{A}{B} = \frac{\text{operating lateral reading} - \text{desired lateral reading}}{B}$$

This would yield the correct angle for each individual varying in a manner dependent on the length B.

5. The point Skull 2 was then marked on the skull using a pencil and the arm of the stereotaxic machine set to the correct angle. The point of the cannula was then positioned onto the point Skull 2 and the vertical reading taken. From this it was possible to lower the cannula 8mm.

Once these calculations had been completed, a small hole was drilled at the relevant point on the skull to allow passage of the cannula. Two further holes were drilled anterior and posterior to this for attachment of stainless steel screws. When the screws were in place the cannula was lowered 8mm, which brought its base level with the skull. It was then attached to the two screws and the skull by means of dental cement (Simplex Rapide, Howmedica International Ltd., London, U.K.). The whole process was then

repeated for the second cannula which was also attached to the first one with dental cement. Care was taken to ensure that the screw heads were completely covered with a smooth layer of cement. The scalp was then sutured with 3/0 chromic suture (Ethicon, Edinburgh, UK) and blank inner 'keepers' inserted into the guides (also 8mm in length). The animals were then given an injection of antibiotic (Ethicilin, Intervet, UK. - 0.2ml i.m.) and placed in an incubator until they had regained consciousness. Recovery was normal for all individuals though there was a tendency for the scalp to withdraw from around the cannulae with time. Iodine solution was applied to the site twice a week until the wounds had completely healed.

3.10 Intracerebral infusion of neuroactive peptides

Males were habituated to handling and infusions immediately preceding pair tests before commencement of the study.

The length of the inner cannula (Clark Electromedical Instruments, Reading, Berks. U.K., od = 0.3mm) was calculated from the measurements taken during surgical implantation of the guide cannulae, taking into account the curvature of the skull between the points Skull 1 and Skull 2. A 1.0 μ l syringe (Dynatech Precision Sampling Co., Baton Rouge, U.S.A.) was attached to these cannulae via silastic tubing (Medical-Grade Tubing, Dow Corning, Michigan, USA - id = 0.28mm). All components of the infusion apparatus were stored in 70% alcohol to maintain sterility before each infusion. The males were hand-held during the infusion which was conducted over 1 minute followed by a 2 minute delay on each side before the cannula was removed. The 'keepers' were then reinserted and the males returned to their cages and left for 5 minutes before commencement of the pair test.

Oxytocin (Sigma, Poole, Dorset, U.K.) was infused bilaterally at 2 doses into each male; 50ng and 100ng contained in 0.5µl sterile physiological saline. Control infusions consisted of bilateral infusions of 0.5µl of the saline vehicle only.

β-endorphin (Sigma, Poole, Dorset, U.K.) was infused bilaterally at 2 doses into each male; 40pmols. and 100pmols. contained in 0.5µl sterile artificial CSF:

NaCl	3.73g	NaHCO ₃	31.76g
KCl	0.19g	NaH ₂ PO ₄ .2H ₂ O	0.18g
CaCl ₂	0.14g	Glucose	0.16g
MgCl ₂ .6H ₂ O	0.19g		

all dissolved in 1litre double distilled water, pH adjusted to 7.3.

Control infusions consisted of bilateral infusions of 0.5µl of the CSF vehicle only.

All solutions were sterilized by passing them through a millipore filter (pore size = 2µm) prior to use and were stored in sterile vials.

3.11 Assay Techniques

Blood samples were collected from the femoral vein of animals during restraint in a tube device to which they been extensively habituated. Samples were collected into heparinized syringes via 25 gauge needles. The blood was then centrifuged at 3,500 rpm for 30 minutes and the plasma stored at -20°C until assayed. Samples of 0.3 - 1.0ml were taken, dependent on the assay requirements.

3.11.1 Testosterone RIA

Plasma samples were assayed for testosterone using the radioimmunoassay technique described by Sharpe & Bartlett (1985). 20 μ l plasma samples were extracted with a 4:1 hexane: diethyl ether mixture. The lower limit of detection was 1ng/ml plasma and the intra-assay coefficient of variation was 10.3% (n=9) using 3 quality controls. All the sequential samples for one animal were measured together in a single assay to avoid problems of inter-assay variation, so the inter-assay coefficient is not relevant.

3.11.2 Oxytocin RIA (carried out by Dr. K. Kendrick, AFRC Babraham.)

Plasma oxytocin concentrations were measured by a radioimmunoassay described previously (Sheldrick & Flint, 1981) using the extraction technique described by Kendrick et al (1986). The limit of detection of the assay was 3 pg/ml plasma and the intra-assay coefficient of variation was 14.5%. All samples were assayed within one assay to avoid problems of inter-assay variation, so the inter-assay coefficient is not relevant.

3.11.3 Progesterone RIA (24 samples assayed by Dr. J. Wickings.)

Plasma levels of progesterone were measured using a radioimmunoassay previously described (Scaramuzzi et al., 1975). 10 μ l samples were used for this assay, the detection limit was 5pg per tube and the intra-assay coefficient of variation was 5%. All samples from each female were measured within one single assay to avoid problems of inter-assay variation, so the inter-assay coefficient is irrelevant.

3.11.4 Luteinizing Hormone Bioassay (13 samples assayed by Dr. S. Lunn)

The concentration of luteinizing hormone (LH) in plasma was measured by

using a mouse Leydig cell bioassay as described previously (Fraser et al., 1980). Diluted plasma samples (1:10, 1:20, 1:40 and 1:80) were assayed in triplicate by incubation with a crude preparation of mouse Leydig cells (~ 150,000 cells per tube) for 3-4 hours at 32°C under a 95% air: 5% CO₂ mixture. The resulting medium was assayed for testosterone using a tritium radioimmunoassay (Sharpe & Bartlett, 1985). The limit of detection of the assay was 2ng/ml, and the intra-assay coefficient of variation was 11.6% (n=30) using 4 quality controls. All the samples shown in this thesis were assayed within the same assay to avoid problems of inter-assay variation, so the inter-assay coefficient is not relevant.

3.12 Histology

3.12.1 Brain tissue

i) Perfusion technique. At the termination of the experiments, a lethal dose of barbiturate (Euthatal, May & Baker, Dagenham, UK - 0.6ml i.v.) was administered. The animals were then perfused through the heart with 60ml heparinized physiological saline followed by 120ml 10% formal saline. The brains were then removed from the skull and left in the fixative for at least one week before subsequent sectioning.

ii) Sectioning and staining. A freezing wedge microtome was used to section brains at 40µm into distilled water. The sections were cut at -18 - -20°C and then stored over night at 4°C before staining. Sections were stained first with 10% luxol fast blue over night at 37°C after being mounted on glycerol-coated slides. This stain was then differentiated using 0.05% Li₂CO₃ and 70% alcohol alternately until the cell nuclei were completely clear and the fibre tracts stained light blue. These sections were then

stained with 1% cresyl fast violet for 10 mins. and differentiated up through the alcohols from 70 - 100% and into xylene. Coverslips were then attached using DPX fixative.

iii) Image analysis. The electrolytic lesions were image analysed using the Imagan2 system (Kompira, Salsburgh, U.K.) on an IBM AT linked to a Zeiss microscope. This package calculated the cross-sectional area of the lesion and, from sequential sections, the volume of each lesion could be calculated.

iv) Anatomical definitions

The medial preoptic area (MPOA) described in the literature is normally considered to consist of the area described in the marmoset brain atlas (Stephan et al., 1980) as the APM - see Fig. 1.3. However, in the present study, the lesions within the preoptic area were centred on the PM - nucleus preopticus medianus, with little or no damage occurring to the APM. To prevent ambiguity, the lesions discussed in this thesis are described as damaging the preoptic area (POA) - including all the nuclei within this area (see Fig. 1.3) - or the preoptic area- anterior hypothalamic continuum (POA-AH), with details of the damage to specific nuclei indicated. This contrasts with the majority of the literature, in which damage is normally confined to the MPOA or the MPOA-AH. When literature is quoted, the definitions of the neural structures in question are those quoted by the authors of the individual papers. Therefore the lesions described in this thesis are not necessarily identical in extent or position to those of other workers and it is hoped that the described difference in nomenclature will make this point clear.

3.12.2 Testicular tissue

Testes were removed from the males after perfusion and fixed in Bouin's

solution. These were subsequently embedded in paraffin wax , sectioned at 5 μ m, and stained using haematoxylin and eosin.

3.13 Statistics

For overall comparisons across individuals, statistics were performed using mean values for each animal. Changes in the frequencies of parameters of male and female behaviours were tested for significance using the Wilcoxon test. Comparisons of pre- and post-operative scores within the same animal were carried out using the Mann-Whitney U-test on the data from each test or day. Statistical advice was sought from a number of sources regarding the use of Wilcoxon tests and Mann-Whitney U-tests in the present context. The consensus of opinion is that these statistical methods are valid under the given conditions (Dr. R.C. Campbell, pers comm.). Furthermore, a non-parametric analysis of variance on the data quoted is thought to be impossible due to the small number of animals in each group, also, this method of analysis would not have led to a different interpretation of the data. Therefore, it appears that the method of analysis employed in this thesis is the only one practicable (Dr. Altham, Statistical Consultant, Cambridge University, pers comm.).

Chi-squared tests were used to analyse the data from the olfactory discrimination tests, and Spearman's rank correlations were employed under a number of conditions to correlate one behaviour type with another within and between animals.

CHAPTER 4

EXPERIMENT 1: POA-AH THERMAL LESIONS AND MASCULINE SEXUAL AND SOCIAL BEHAVIOUR IN A PAIR TEST SITUATION

4.1 Introduction

The MPOA-AH is intimately involved in the control of the masculine sexual behaviour of vertebrates (see Chapter 2), though relatively little work has been carried out to elucidate its role in primates. It was decided that a study employing a thermal lesion technique, should be undertaken to provide a foundation upon which to base future work employing more discrete neurophysiological techniques in the common marmoset.

Furthermore, there is clearly a dispute within the literature on the question of the precise role of the MPOA-AH. There is evidence to implicate this area in the control of sexual arousal, and equally compelling evidence to indicate that the MPOA-AH is also concerned with the control of copulatory behaviour. It was believed that, by studying a wide range of the social and sexual behaviours of the common marmoset, this question could be addressed in greater detail than in some previous studies. It was also hoped that, if these males still experienced sexual arousal post-operatively, then by providing an increased stimulus in the form of an oestrogen-treated proceptive female, some increase in components of their arousal would be apparent.

A further point of interest was whether or not the males' relationship with other males was affected by these lesions, and for this reason, aggression tests were carried out between an experimental male and an unknown 'intruder' male.

4.2 Materials and methods

4.2.1 Animals

The 26 adult males and 14 adult females used in this study were selected using the criteria outlined in Chapter 3. The females were ovariectomized prior to commencement of the study but received hormone replacement treatment only during one phase of the study as described below.

4.2.2 Surgery

Males received thermal hypothalamic lesions (n=10) or sham lesions (n=4) as described in section 3.9.3 of Chapter 3. The atlas coordinates used were as follows: AP +10.0mm, V +7.5mm + +6.8mm, L \pm 0.5mm (n=7), AP +10.0mm, V +7.5mm + +8.5mm, L \pm 0.5mm (n=1) and AP +10.3mm, V +8.0mm + +9.0mm, L \pm 0.5mm (n=2) for the lesioned males and AP +10.7mm, V +8.5mm, L \pm 0.5mm (n=4) for the sham lesioned controls. Testing resumed 7-14 days after surgery.

4.2.3 Testing regime

Each pair was tested 2-3 times per week between 10.00h and 16.00h for 30 minutes. All males received 20 pre-operative and 20 post-operative tests except for male no. 9 who only had 16 post-operative tests due to ill health. At the end of the post-operative test series, a further 5 tests were carried out in 11 cases (male nos. 1, 3, 4, 5, 6, 7, 8, C1, C2, C3 and C4) to investigate specifically the reactions of the males to the proceptive displays shown by their females. After these 5 tests, the female partners of these 11 males received 2 silastic oestrogen implants to stimulate their proceptive behaviour (see Chapter 3). A further 5 tests were then carried out commencing 5 days after the implants were administered.

12 males were also tested for the occurrence of agonistic behaviour when faced with a strange male (male nos. 2 - 8, 10, C1 -C4). In this instance, the test apparatus was assembled as usual, but a strange male was substituted for the males' familiar female partner. Tests commenced from entry of the intruder into the males' home cage and the 15 minute test was divided into 30 second blocks. The presence of agonistic or submissive behaviour for either of the males was recorded during each 30 second interval such that absolute occurrences of a particular behaviour were not scored. The test was terminated immediately if undue aggression occurred. Each of these 12 males received 1 pre-operative test (conducted after termination of the pre-operative pair test series immediately prior to surgery) and 1 post-operative test (conducted at the conclusion of the study immediately prior to euthanasia). The same male acted as intruder for the pre- and post-operative test.

4.2.4 Behaviours scored

The specific behaviours listed here are defined in detail in Chapter 3.

During the pair tests the following behaviours were scored for the male; anticipatory penile erection, precopulatory tongue flicks, anogenital investigation of the female, mount attempts, mounts, mount latency, intromissions, intromission latency, ejaculations, ejaculation latency, whether the mount included an erection and/or pelvic thrusting, self-anogenital investigation, allogrooming, grooming invitations, scent marking, genital presents and aggression. For the female the following were scored; proceptive bouts, mount refusals, mount terminations, whether or not the mount was initiated by her, allogrooming, grooming invitations, and aggression.

In the case of the oestrogen treatment phase, the males' reaction to the

females' entry into the cage and subsequent proceptive displays was scored. It was noted whether the male and female had eye contact during proceptive bouts and whether the male responded to these bouts by precopulatory tongue flicks, approaching the female, anogenital investigation of the female, a grooming invitation, allogrooming, a hip touch, or a mount. Any copulatory behaviour occurring during these 30 minute tests was scored in the same way as that seen in a standard pair test. The occurrence of these behaviours was also scored if they occurred in the absence of cues from the female. For the female, it was noted whether each proceptive bout consisted of just immobility and a stare, or whether tongue flicking occurred simultaneously with these other proceptive displays. Behaviours scored during the aggression tests consisted of all agonistic behaviours shown by both the resident (lesioned or sham lesioned male) and the intruder ('strange' male).

4.2.5 Blood sampling and assay

Blood samples were taken from the femoral vein of both the lesion and sham-lesion animals at weekly intervals for subsequent determination of plasma testosterone levels by radioimmunoassay (see Chapter 3). 0.4ml samples were collected from each male between 09.00h and 10.00h on a day when the animals were not being pair tested.

4.2.6 Histology

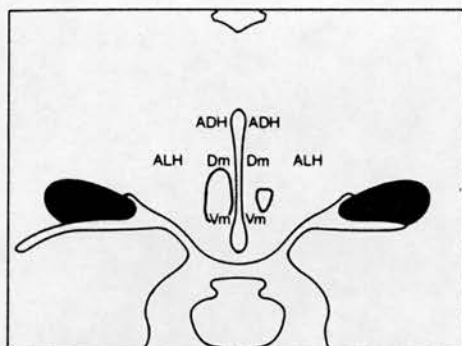
The brains of the thermally lesioned males were prepared and treated in the manner described for staining with luxol fast blue and cresyl fast violet in Chapter 3. Lesion volumes were then calculated using the image analyser. Testes were also collected from each lesioned male and prepared as described in Chapter 3.

4.3 Results

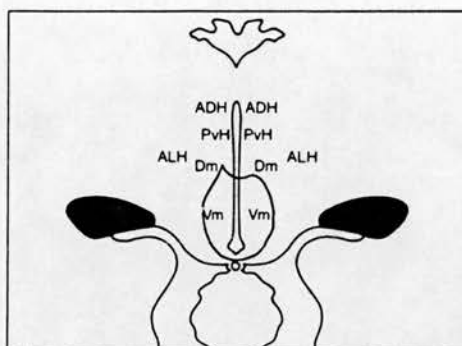
The method of statistical analysis chosen for this data was determined by the variation in lesion placement between the 10 males. Wilcoxon tests were carried out across males to investigate the generalised effects of lesions within the anteromedial hypothalamus and preoptic area on sexual and social behaviour. However, in order to highlight differences in the effects of these lesions between individuals, Mann-Whitney U-tests were carried out within males. These differences in effect may have been due to differences in lesion placement within this broad area.

4.3.1 Lesion placement

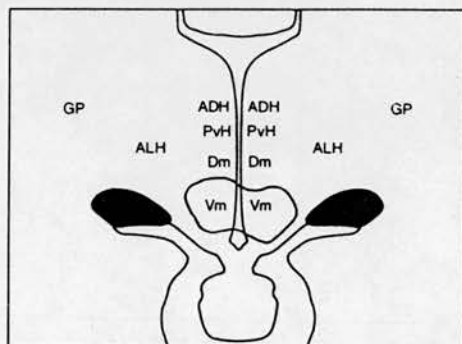
Fig. 4.1 shows a series of frontal sections of the lesions in each male. Here, and subsequently, the animals are numbered 1-10 in order of lesion placement relative to interaural zero i.e. male no. 1 has the most caudal and male no. 10 the most rostral lesion placement. There was a wide range of lesion positions and Fig. 4.2 shows the nuclei damaged in each male. Male nos. 1 and 2 had lesions centred in the ventromedial hypothalamus though, in the case of male no. 2, the damage was unilateral. In male nos. 6, 7, 8, and 9, there was major damage to at least one nucleus in the preoptic area (POA) - mainly the nucleus preopticus medianus (PM). In 5 individuals (male nos. 3, 4, 5, 6, and 7) lesions were centred in the anterior hypothalamus under the anterior commissure at the level of the paraventricular and dorsalis anterior nuclei though, in the case of male no. 3, the damage was unilateral. In male no. 10 the lesions were rostral to the POA affecting mainly the diagonal band of Broca. Fig. 4.2 also shows the approximate AP coordinate of the lesion centre in each male (relative to interaural zero) and the lesion volumes, which varied from 1.49 - 3.28 mm³.



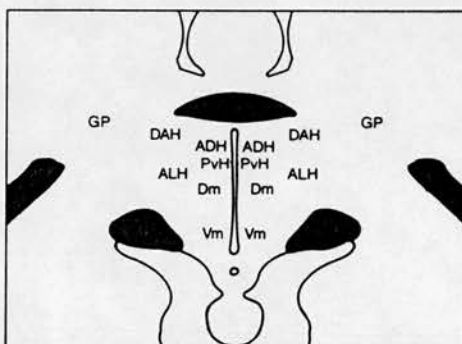
AP 7.5



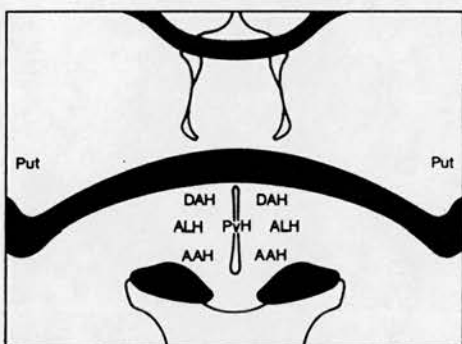
AP 8



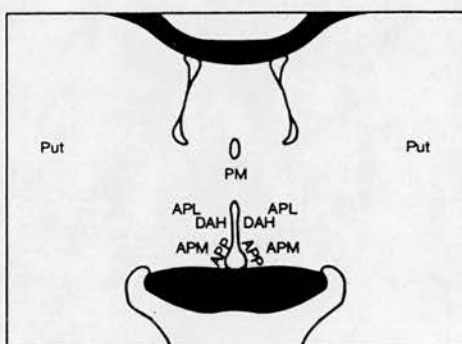
AP 8.5



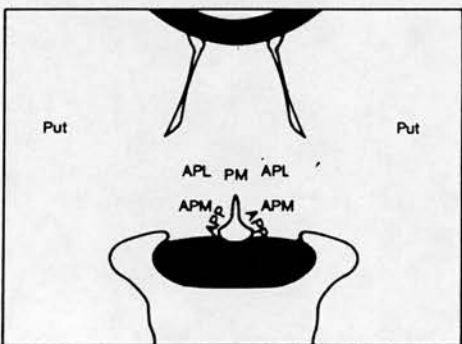
AP 9



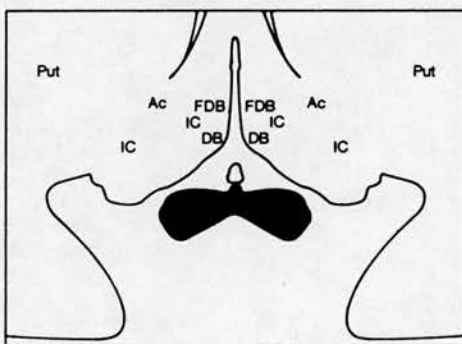
AP 9.5



AP 10

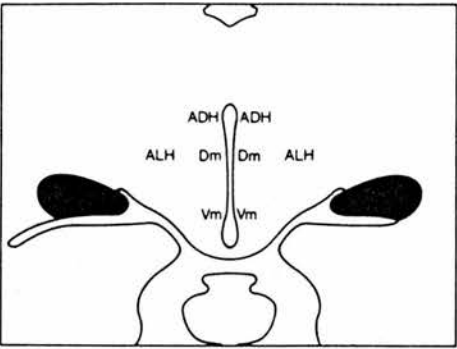


AP 10.5

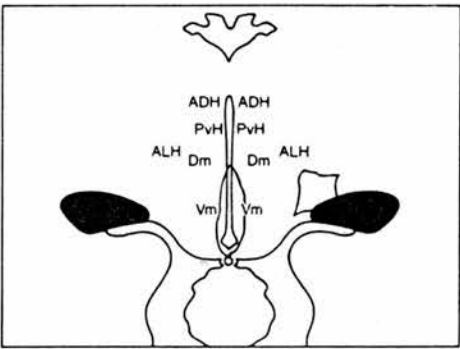


AP 11.5

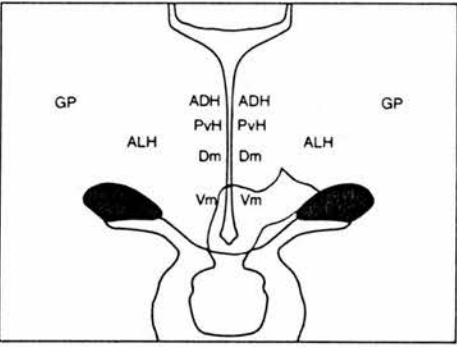
Male No. 1



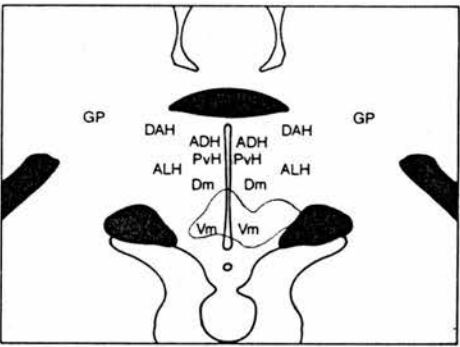
AP 7.5



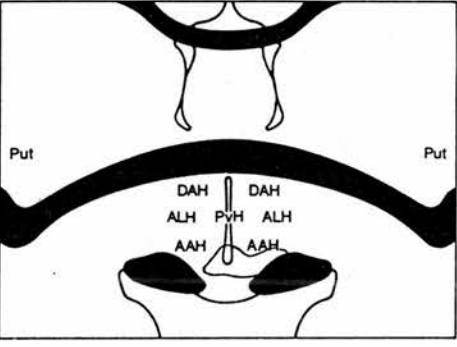
AP 8



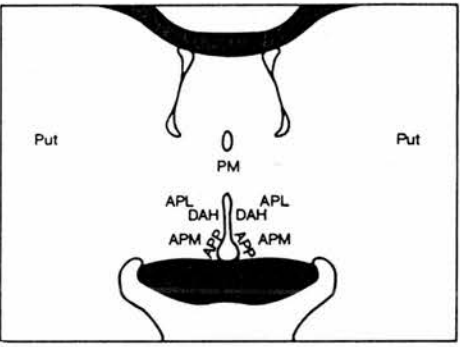
AP 8.5



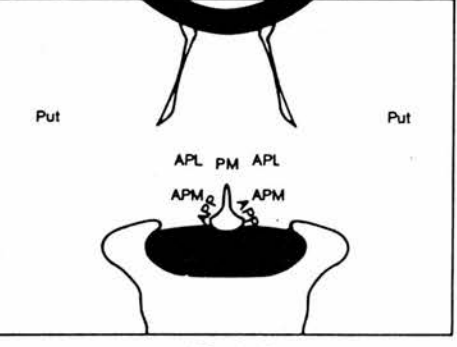
AP 9



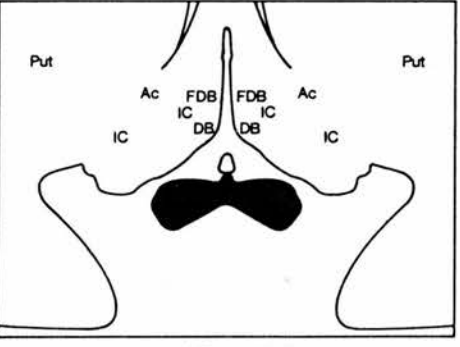
AP 9.5



AP 10

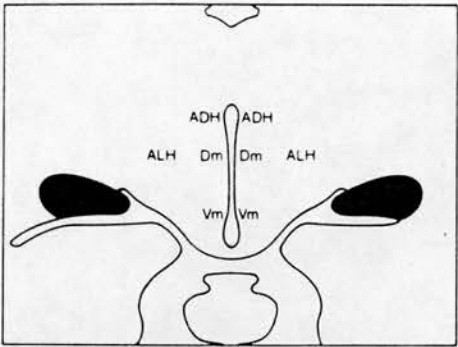


AP 10.5

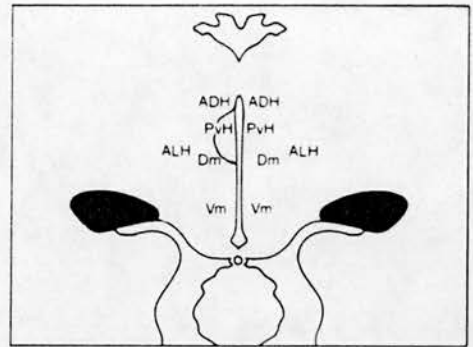


AP 11.5

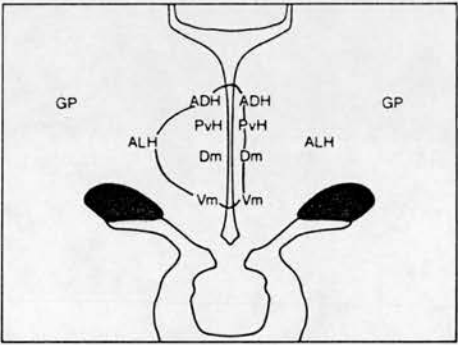
Male No. 2



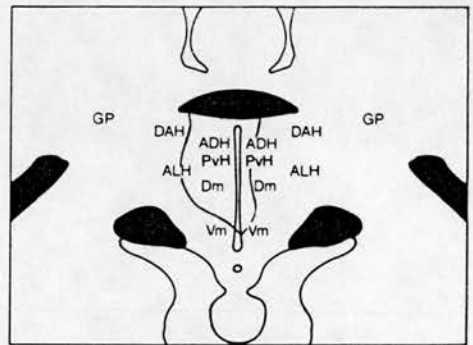
AP 7.5



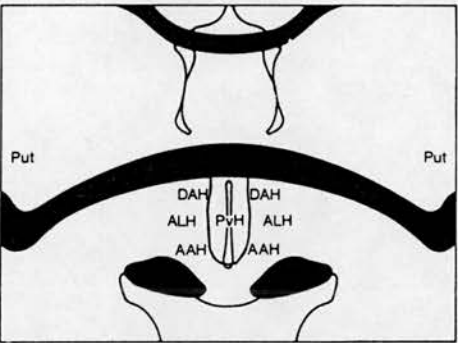
AP 8



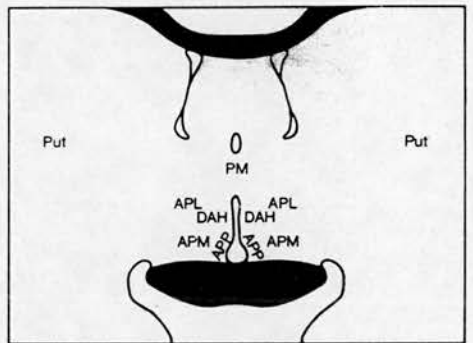
AP 8.5



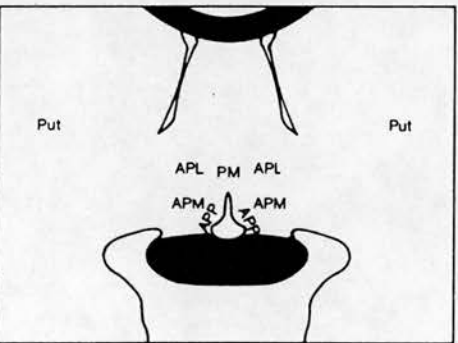
AP 9



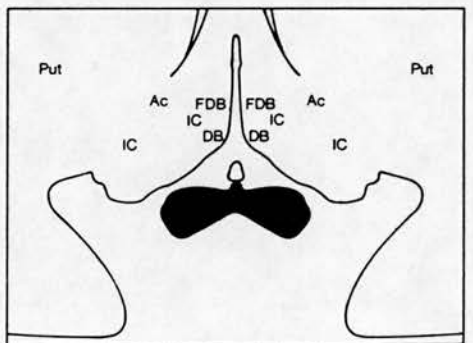
AP 9.5



AP 10

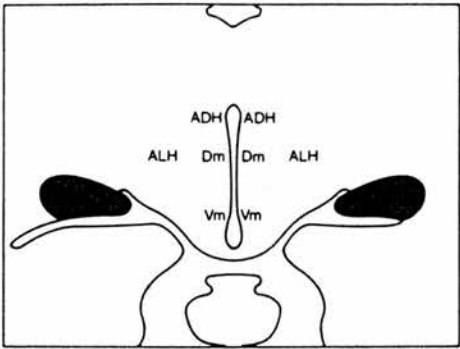


AP 10.5

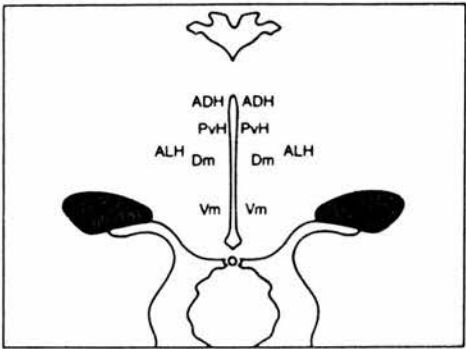


AP 11.5

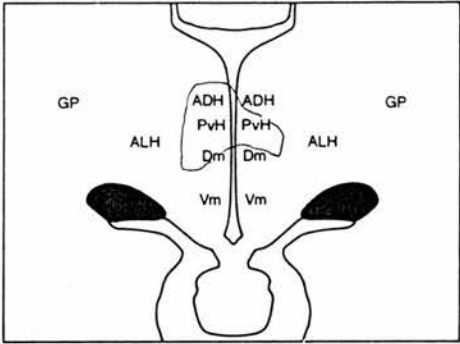
Male No. 3



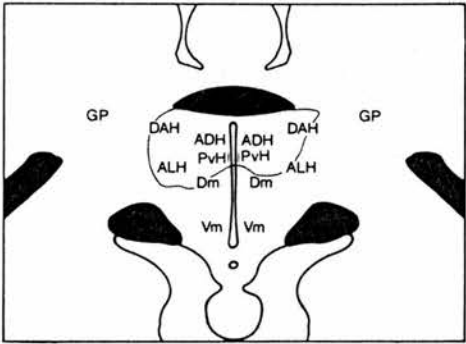
AP 7.5



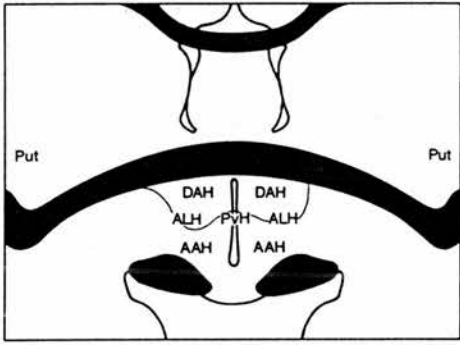
AP 8



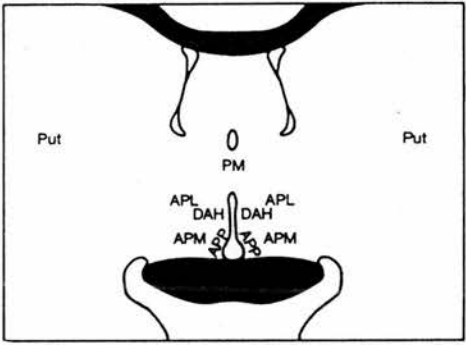
AP 8.5



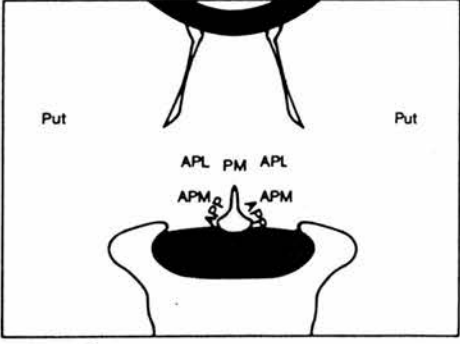
AP 9



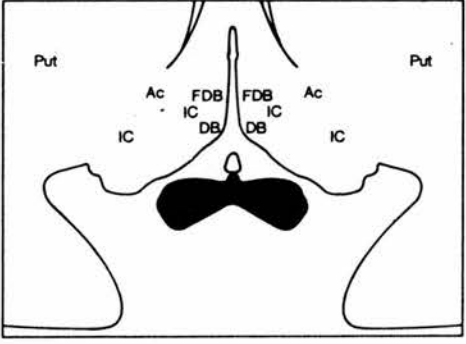
AP 9.5



AP 10

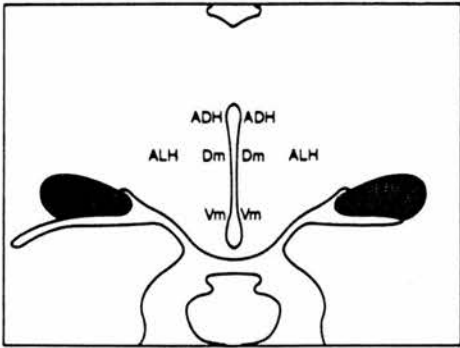


AP 10.5

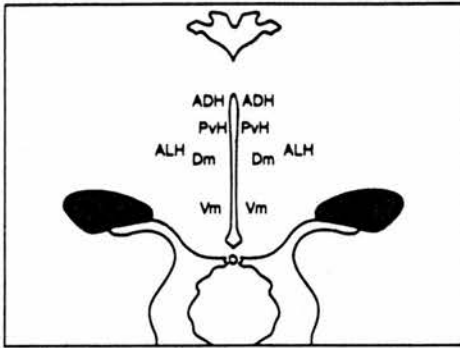


AP 11.5

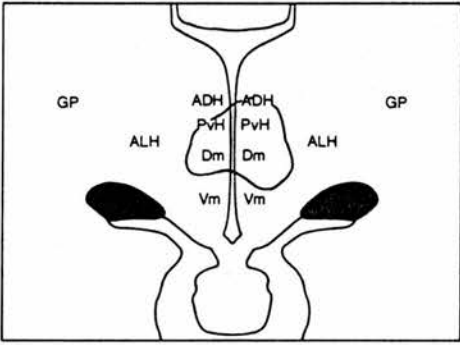
Male No. 4



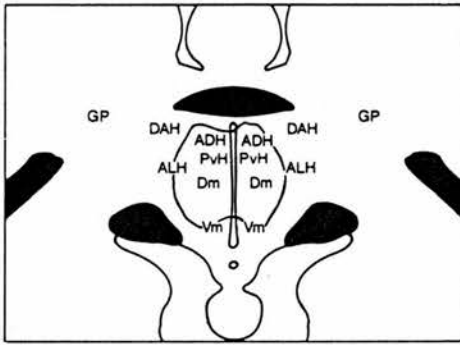
AP 7.5



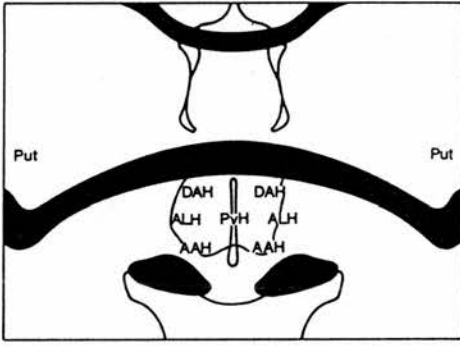
AP 8



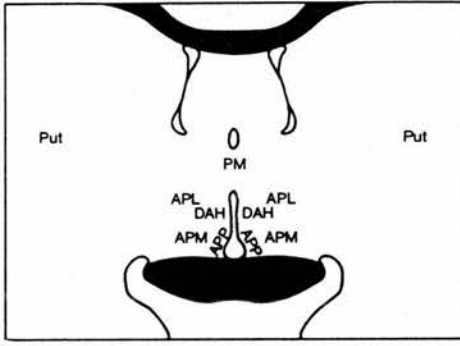
AP 8.5



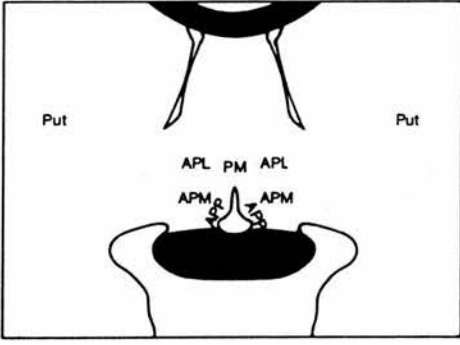
AP 9



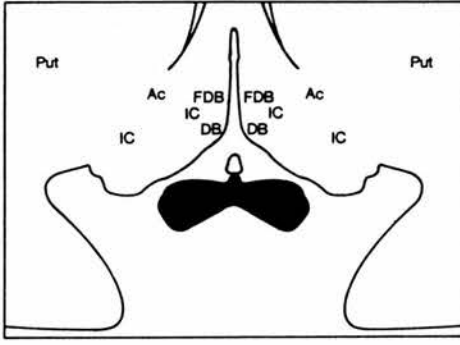
AP 9.5



AP 10

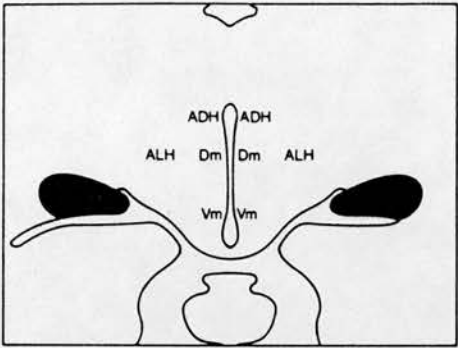


AP 10.5

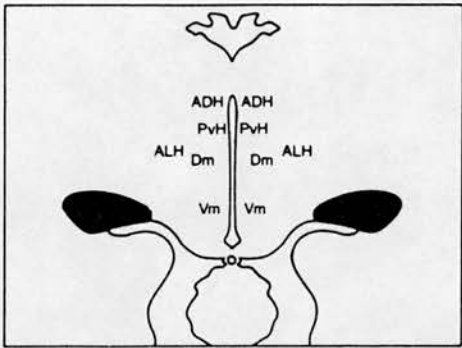


AP 11.5

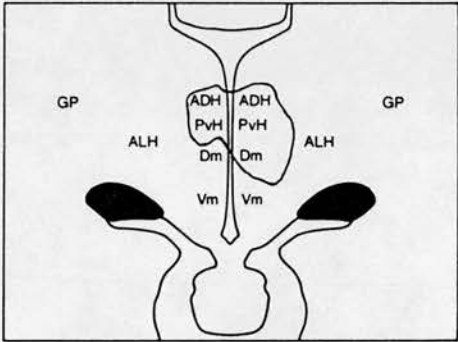
Male No. 5



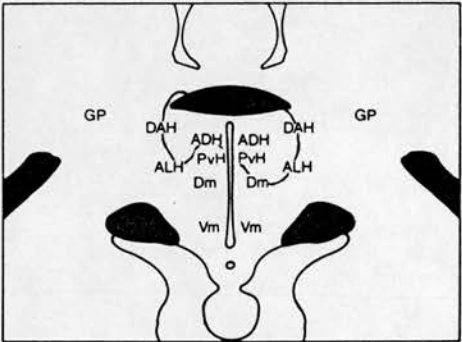
AP 7.5



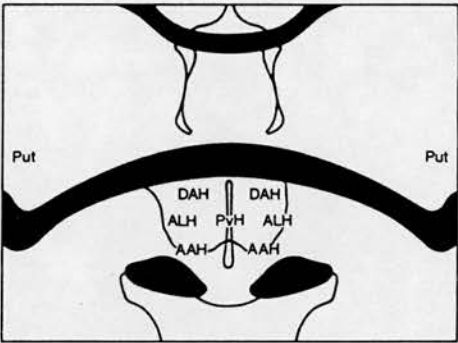
AP 8



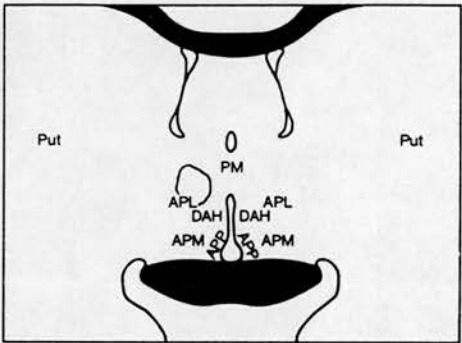
AP 8.5



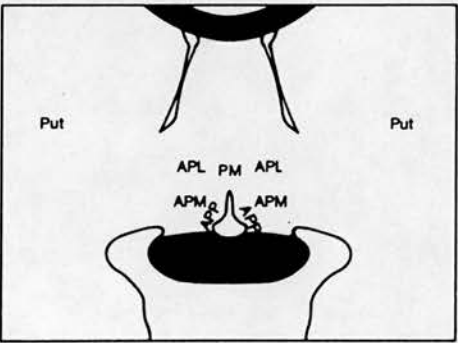
AP 9



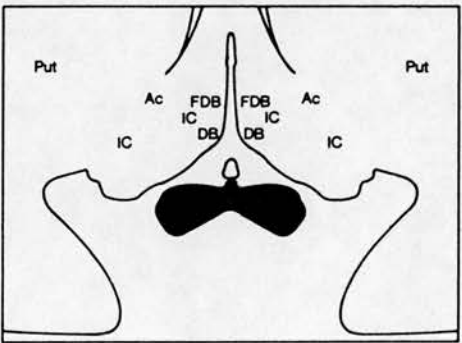
AP 9.5



AP 10

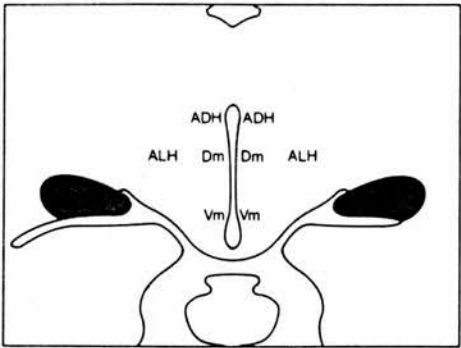


AP 10.5

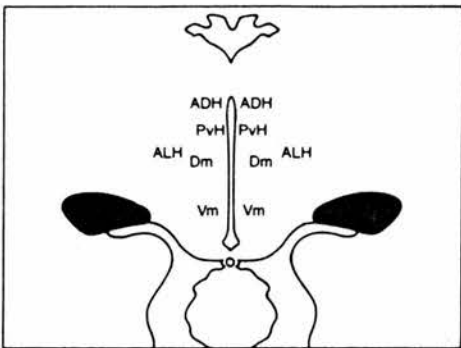


AP 11.5

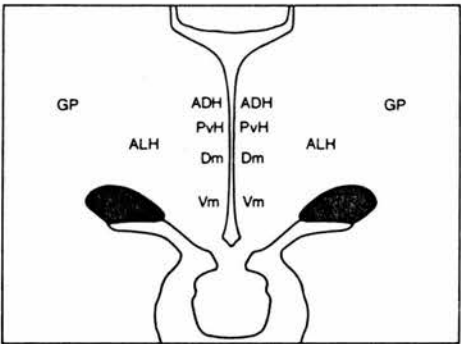
Male No. 6



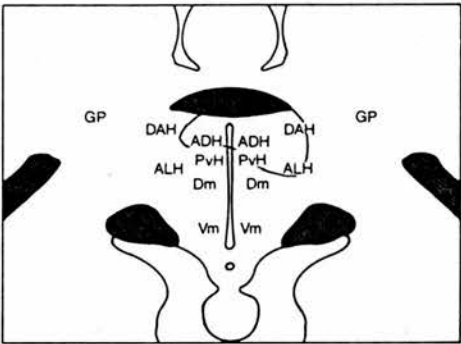
AP 7.5



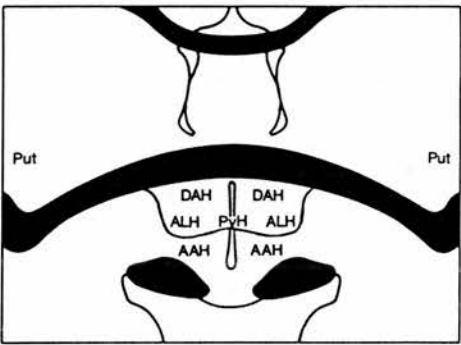
AP 8



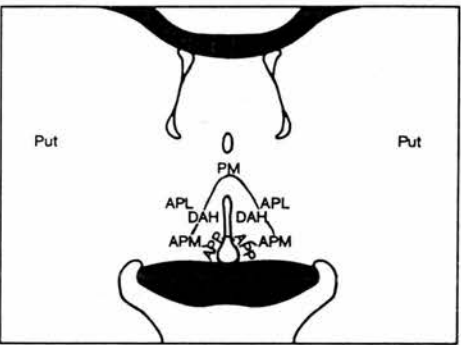
AP 8.5



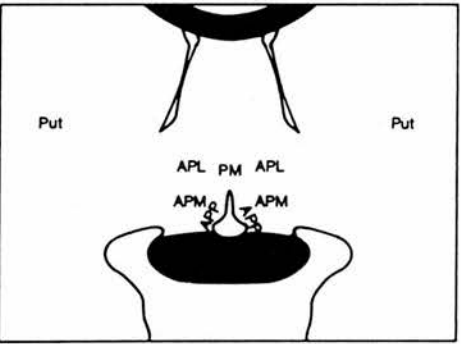
AP 9



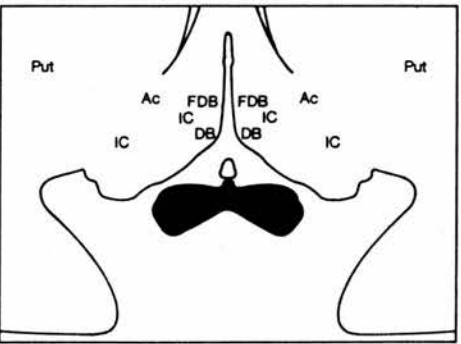
AP 9.5



AP 10

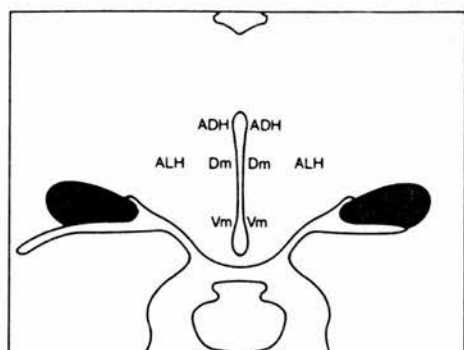


AP 10.5

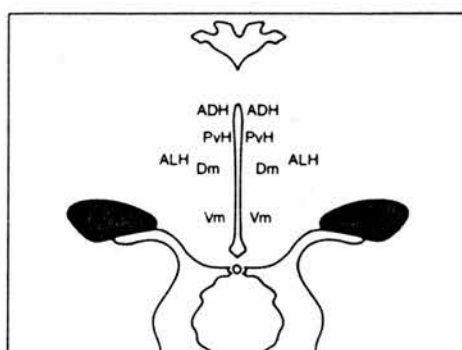


AP 11.5

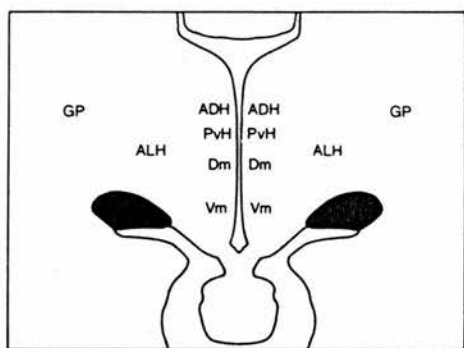
Male No. 7



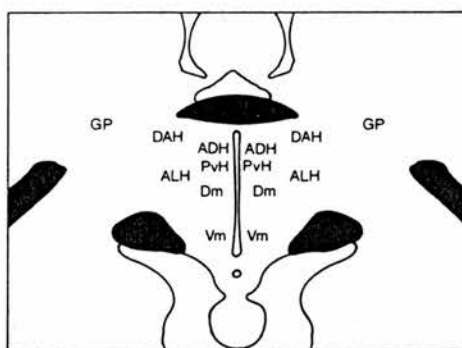
AP 7.5



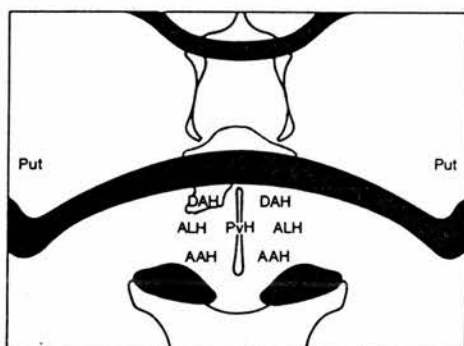
AP 8



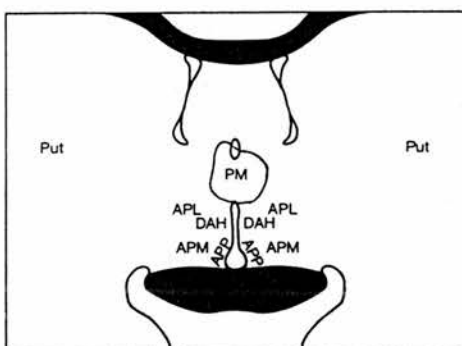
AP 8.5



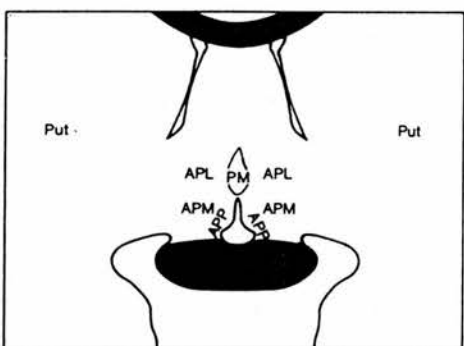
AP 9



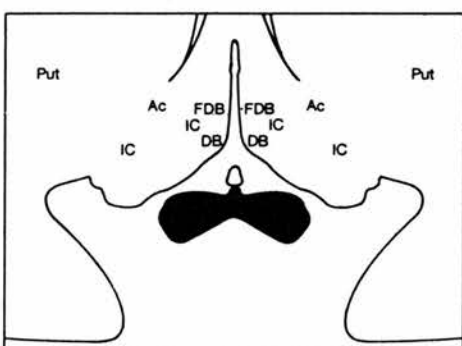
AP 9.5



AP 10

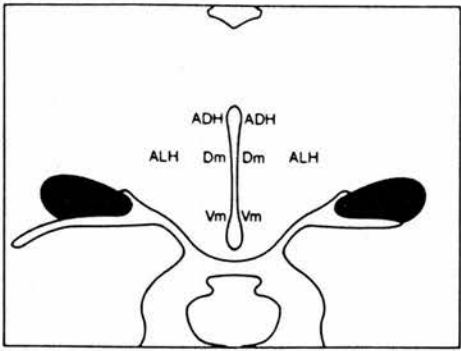


AP 10.5

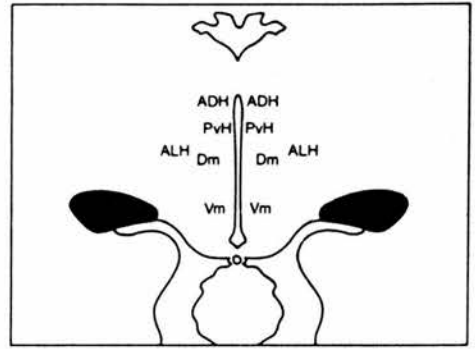


AP 11.5

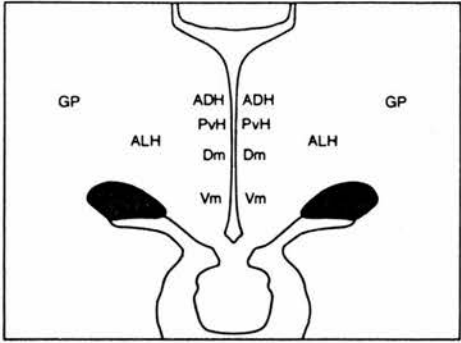
Male No. 8



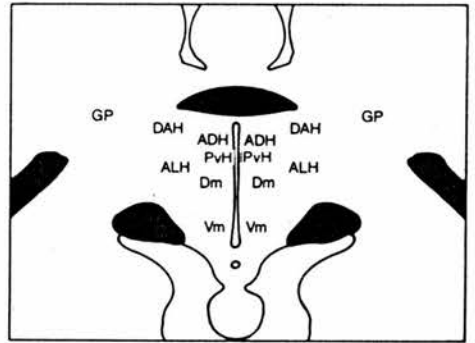
AP 7.5



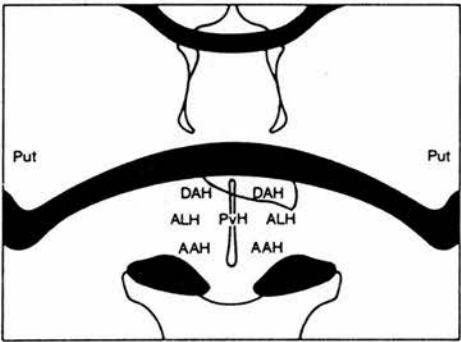
AP 8



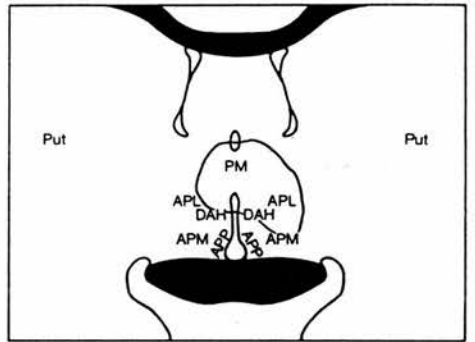
AP 8.5



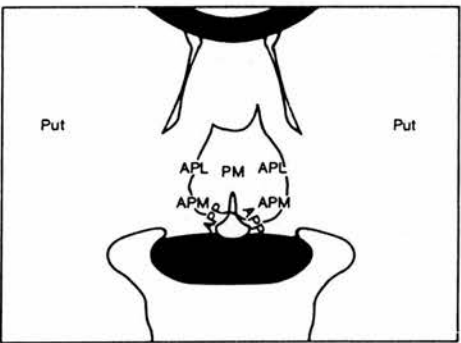
AP 9



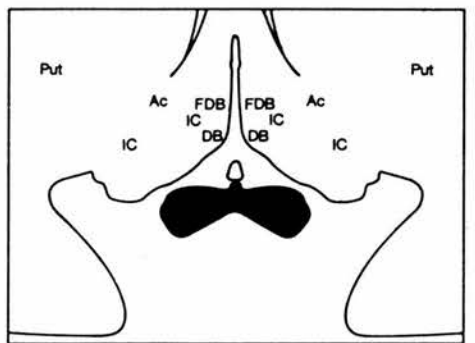
AP 9.5



AP 10

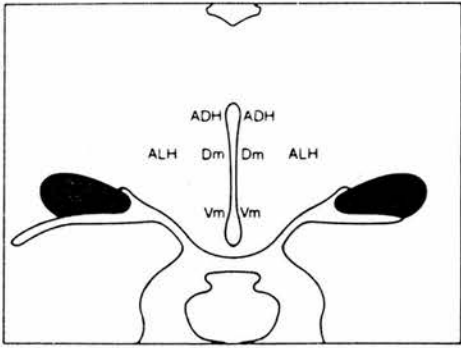


AP 10.5

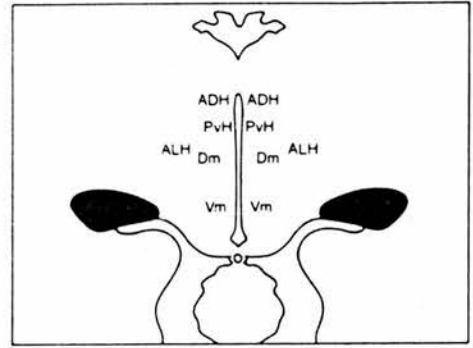


AP 11.5

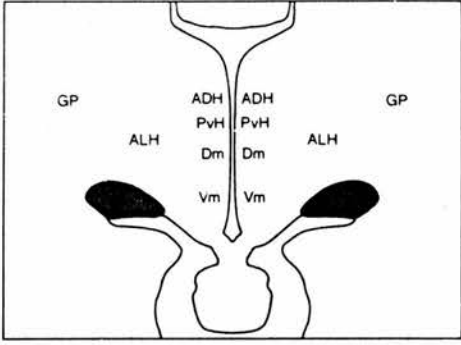
Male No. 9



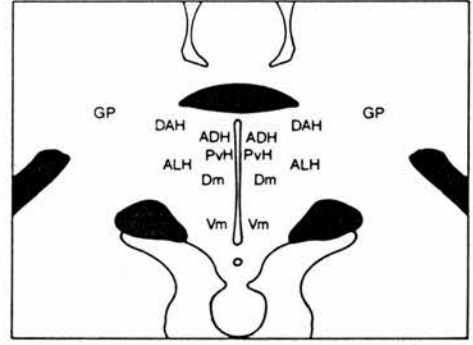
AP 7.5



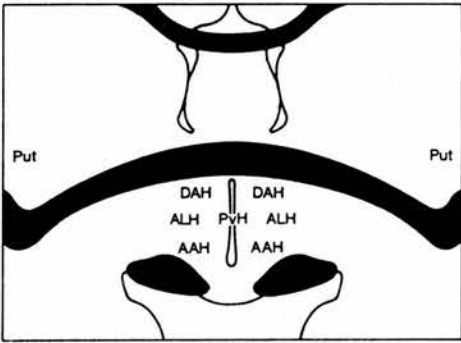
AP 8



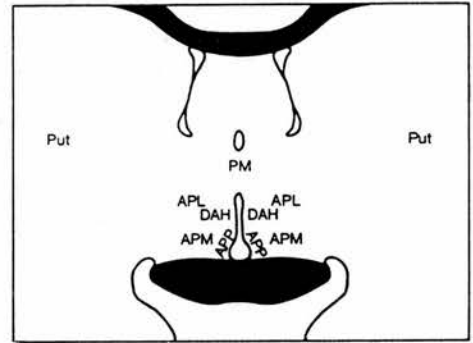
AP 8.5



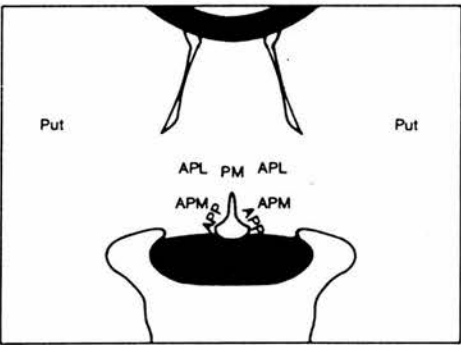
AP 9



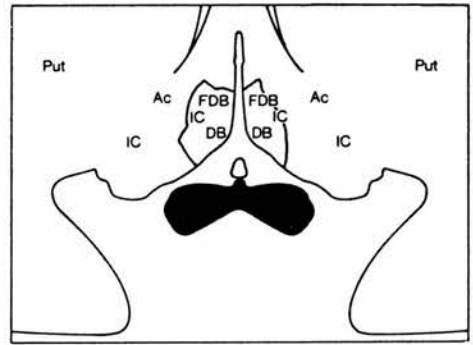
AP 9.5



AP 10



AP 10.5



AP 11.5

Male No. 10

Fig. 4.1 The position and extent of the lesions of male nos. 1-10.

The lesions from each of the males are shown on a series of sections (AP 7.5mm - 11.5mm) taken from the marmoset atlas (Stephan et al., 1980). All sections are shown for each male to facilitate comparison of lesion placement between the 10 individuals.

Key:-

Vm = ventromedial nucleus
Dm = dorsomedial nucleus
ALH = lateral hypothalamic area
ADH = dorsal hypothalamic area
PvH = paraventricular nucleus
GP = globus pallidus
DAH = dorsalis anterior nucleus
AAH = anterior hypothalamic area
Put = putamen
APP = preoptic periventricularis nucleus
APM = medial preoptic area
APL = lateral preoptic nucleus
PM = nucleus preopticus medianus
DB = diagonalis Brocae
IC = insulae Callejae
FDB = fasciculus diagonalis Brocae
Ac = nucleus accumbens

	OTHER			POA				AH				OTHER			AP AT CENTRE	VOLUME (mm3)
	DB	IC	FDB	PM	APL	APM	APP	DAH	ALH	AAH	PvH	ADH	Vm	Dm		
1											*		**	*	8.4	2.41
2										*			**		8.6	2.25
3								**	*	*	***	**	*	**	8.8	2.64
4				*	*			***	*		**	**		*	9.2	3.06
5						*		**	*	**	***	***	*	***	9.3	3.28
6					*			**	*	*	**	***		*	9.5	2.71
7				*	*	*		**	*		*	*			9.6	1.49
8				***	*			*							9.9	2.41
9				***	**	*	*	*							10.5	3.20
10	**	**	**												11.0	1.64

Fig. 4.2 Positions and volumes of hypothalamic lesions in male marmosets (nos. 1-10).

The degree of damage to the individual nuclei is shown, as well as the position of the lesion centre relative to interaural zero (AP), and the total volume of tissue damaged (mm³).

* = slight damage only ** = medium damage *** = substantial damage

DB = diagonalis Brocae

IC = insulae Callejae

FDB = fasciculus diagonalis Brocae

PM = nucleus preopticus
medianus

APL = lateral preoptic nucleus

APM = medial preoptic area

APP = preoptic periventricularis nucleus

DAH = dorsalis anterior nucleus

AAH = anterior hypothalamic area

ALH = lateral hypothalamic area

PvH = paraventricular nucleus

ADH = dorsal hypothalamic area

Vm = ventromedial nucleus

Dm = dorsomedial nucleus

4.3.2 Effects on sexual behaviour

4.3.2.1 Sexual arousal

The effects of lesions on frequencies of precopulatory behavioural patterns displayed by individual males are shown in Fig. 4.3. Wilcoxon tests (in each case comparing the mean pre- and post-operative scores) revealed an overall decrease in frequencies of anticipatory erections (from mean + s.e.m. 68.0 +- 11.4% to 15.5 +- 8.25% of tests, T=2.5, n=10, P<0.01),

anogenital investigation of females (from 3.33 ± 0.66 to 1.7 ± 0.4 per test, $T=2$, $n=9$, $P<0.01$) and bouts of precopulatory tongue flicking (from 1.19 ± 0.36 to 0.35 ± 0.12 per test, $T=7$, $n=10$, $P<0.05$) by the 10 males after hypothalamic lesions. Individual variations in effects of lesions on behaviour were pronounced. Mann-Whitney U-tests within individuals showed that 6 males exhibited statistically significant decreases in frequencies of anticipatory erections (male nos. 3,5,6,7,9 and 10), whilst anogenital investigations of the females decreased significantly in 5 males (nos. 1,6,7,9 and 10). Precopulatory behaviour was not decreased by sham lesions in the control males C1-C4, and male C4 showed an overall increase during post-operative tests (see Fig. 4.3). Although male C1 exhibited less tongue flicking behaviour after a sham lesion, this was not due to the operation, but represented a continuation of a gradual decline which began during the pre-operative test series (see Fig. 4.4). During the first and second blocks of 10 pre-operative tests, tongue flicks decreased from 147 ± 27.8 to 44.1 ± 7.6 per test ($U=7$, $n=10$, $P<0.001$) in male C1. However, frequencies of tongue flicking in this male were not statistically different when the 10 tests immediately before and after the sham lesion were compared. The same finding applied to lesioned male nos. 5 and 6. The gradual decreases in the frequencies of precopulatory tongue flicking in these males possibly reflects effects of habituation due to repeated testing with the same females. However, in the remaining 2 cases (male nos. 7 and 9) there were abrupt decreases in tongue flicking coincident with surgery (see Fig. 4.4), and comparison of the 2nd 10 pre-operative and 1st 10 post-operative tests yielded significant effects (male no. 7: $P<0.01$ and male no. 9: $P<0.001$). A note of caution is that male no. 9 exhibited weight loss and lowered body temperature subsequent to lesioning, and male no. 7 was the only animal that showed a significant decline in plasma testosterone levels (see below).

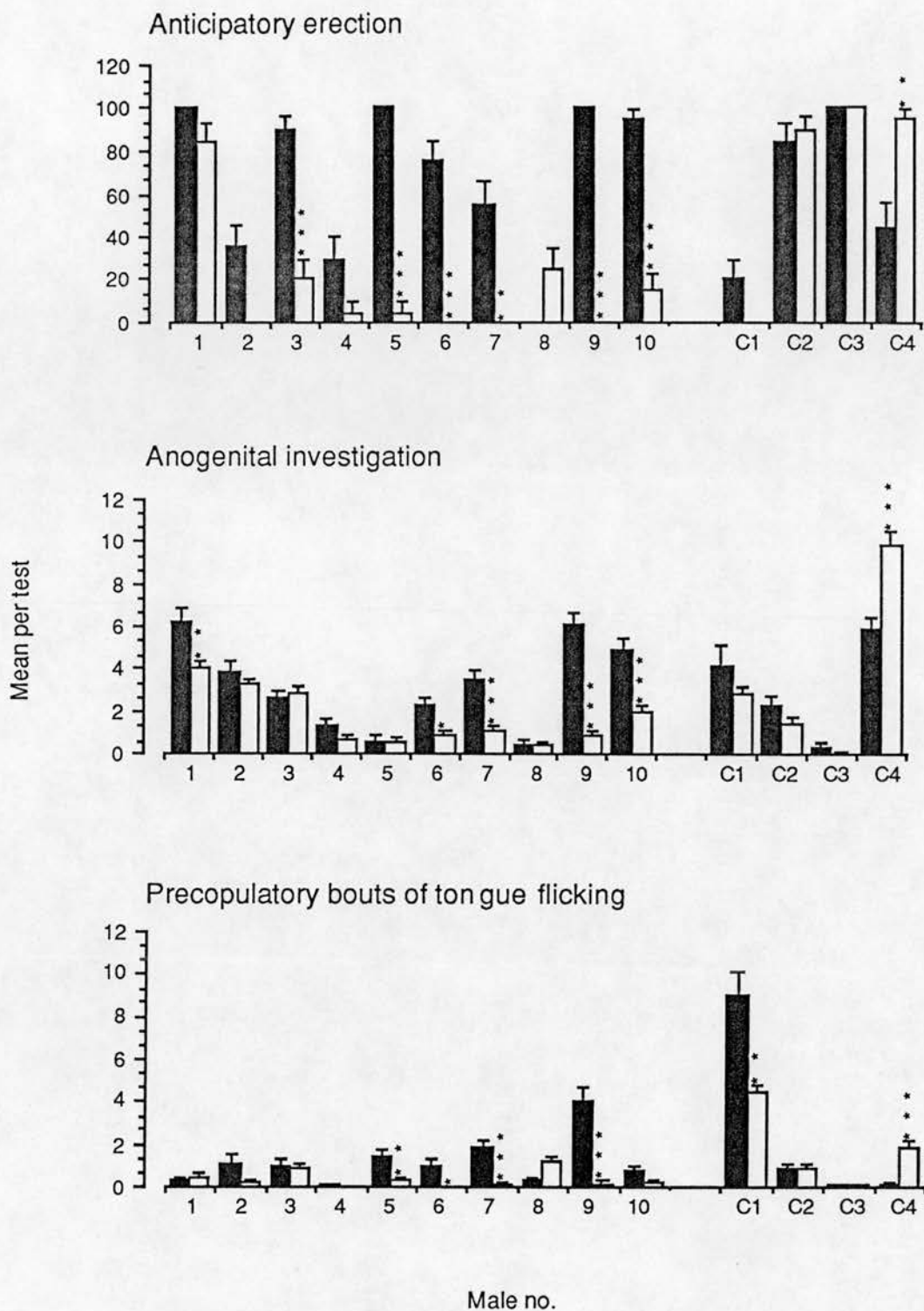


Fig. 4.3 Effects of lesions (males 1-10) or sham lesions (males C1-C4) on arousal behaviour.

Histograms show mean \pm s.e.m. frequencies per test for each male pre- (solid bar) and post-lesion (open bars). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, Mann-Whitney U-test (within-animal comparisons).

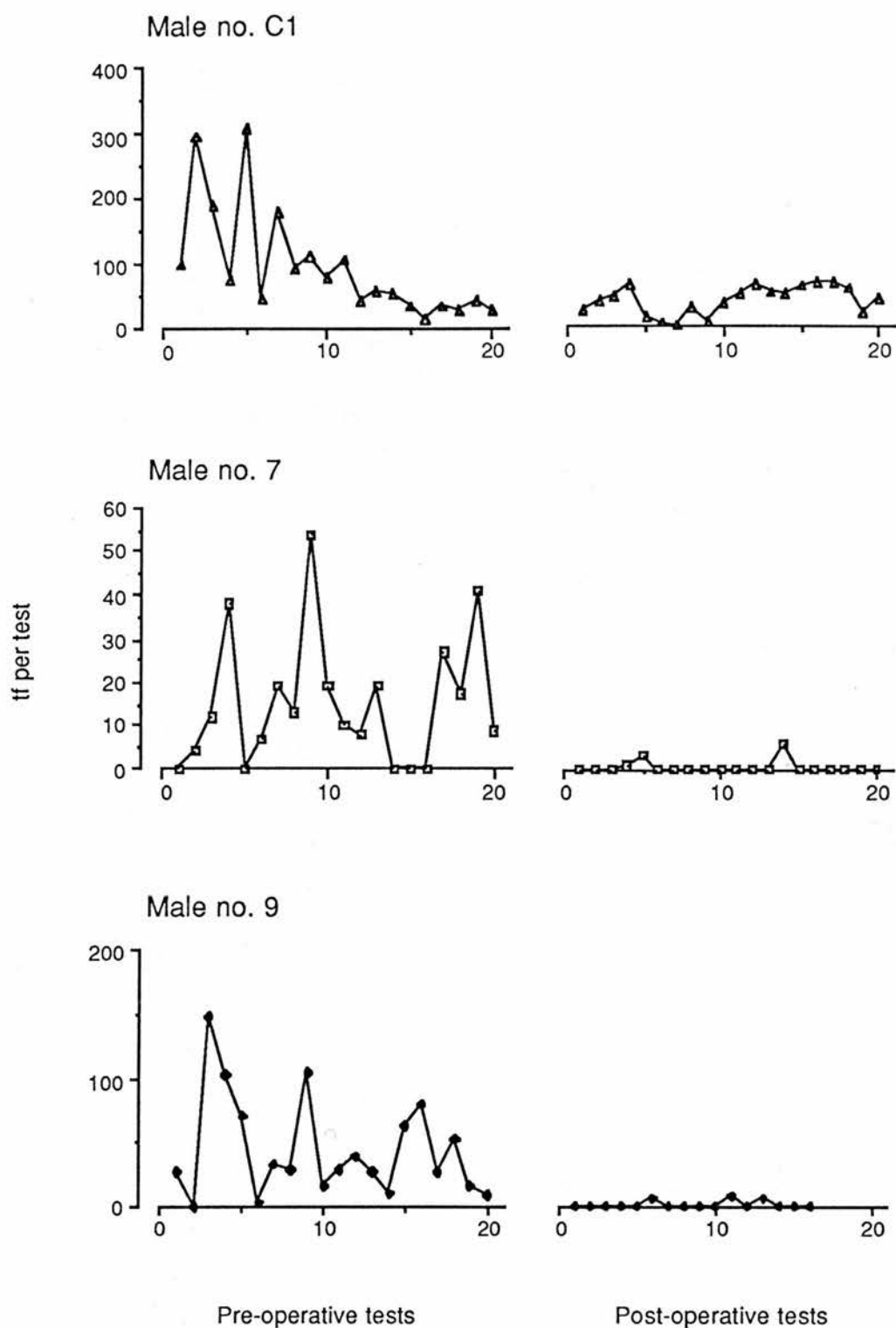


Fig. 4.4 Effects of a sham lesion (male C1) and lesions (males 7 + 9) on tongue flicking behaviour.

Histograms show mean \pm s.e.m. frequencies per test for each male pre- (solid bar) and post-lesion (open bars). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, Mann-Whitney U-test (within-animal comparisons).

4.3.2.2 Copulatory behaviour

Fig. 4.5 shows the effects of lesions on frequencies of mounting, intromission and ejaculation by individual males. Wilcoxon tests (in each case comparing the mean pre- and post-operative scores) revealed an overall decrease in frequencies of mounts (from mean \pm s.e.m. 3.92 ± 0.54 to 1.52 ± 0.38 per test, $T=1$, $n=10$, $P<0.01$), intromissions (from 1.52 ± 0.12 to 0.54 ± 0.18 per test, $T=0$, $n=10$, $P<0.01$) and ejaculations (from 1.36 ± 0.06 to 0.51 ± 0.18 per test, $T=0$, $n=10$, $P<0.01$) in the 10 lesioned males. As with pre-copulatory behaviour, it is important to consider data for individuals as there was a wide range a lesion positions and volumes and therefore the 10 males are not a truly homogeneous group. However, all the males, except no. 8, exhibited statistically significant decreases in mount and intromission frequencies, whilst ejaculations were significantly less frequent in all lesioned males except nos. 8 and 10. Interestingly, male no. 8, which had sustained a lesion of 2.41mm^3 in the POA, showed no significant decrease in any aspect of arousal or copulatory behaviour; whereas in male no. 9, in which POA damage encompassed an area of 3.20mm^3 , significant reductions in all behavioural measurements occurred (Figs. 4.3 and 4.5). However, interpretation of lesion effects in male no. 9 is complicated by the fact that behaviour during the post-operative testing series may have been affected by ill health (see below).

A comparison of Fig. 4.5 and Fig. 4.2 indicates that the most dramatic effects upon all measurements of copulatory behaviour occurred in 4 males (nos. 4,5,6 and 7) in which the lesions were situated bilaterally below the anterior commissure at the level of the dorsalis anterior and paraventricular nuclei. Males with more rostral or caudal lesions were not as severely affected. Male no. 1, in which the VmH was extensively damaged, continued to mount at a mean frequency of 1.75 ± 0.22 per test. However, this male attained

intromission during only 17.1% of these post-operative mounts. On the 6 occasions upon which intromission occurred, the male ejaculated 4 times indicating that this component of copulatory behaviour was still functional.

Data on mount, intromission and ejaculation latencies were analysed statistically for those males which continued to exhibit these behavioural patterns at sufficient frequencies after hypothalamic lesions. The results, along with the data on sham lesioned controls are shown in Fig. 4.6. Male nos. 4,6 and 7 mounted too infrequently after lesions for statistical analysis of latencies, and in the remaining males, latencies were significantly increased in male nos. 1,2,3,5 and 9. 4 lesioned males continued to intromit and ejaculate at sufficient frequencies to perform Mann-Whitney U-tests within animals by comparison with pre-operative data. Male nos. 2,9 and 10 exhibited increases in intromission latencies after lesioning, but in only 1 case (male no.9) was ejaculation latency significantly affected (Fig. 4.6). Among the sham lesioned controls, no effects upon mount latency or ejaculation latency occurred, but intromission latency was significantly increased in male C2 and significantly decreased in male C4 during the post-operative test series.

There were no consistent effects of lesions upon penile erection or occurrence of pelvic thrusting during mounting in male marmosets (Fig. 4.7). The percentage of male's mounts initiated by females continued at similar frequencies, except in the case of male no. 5, in which a significant reduction in female-initiated mounts occurred (Fig. 4.7). Mount refusals and terminations were so infrequent both pre- and post-operatively that no statistical analysis of the data was possible.

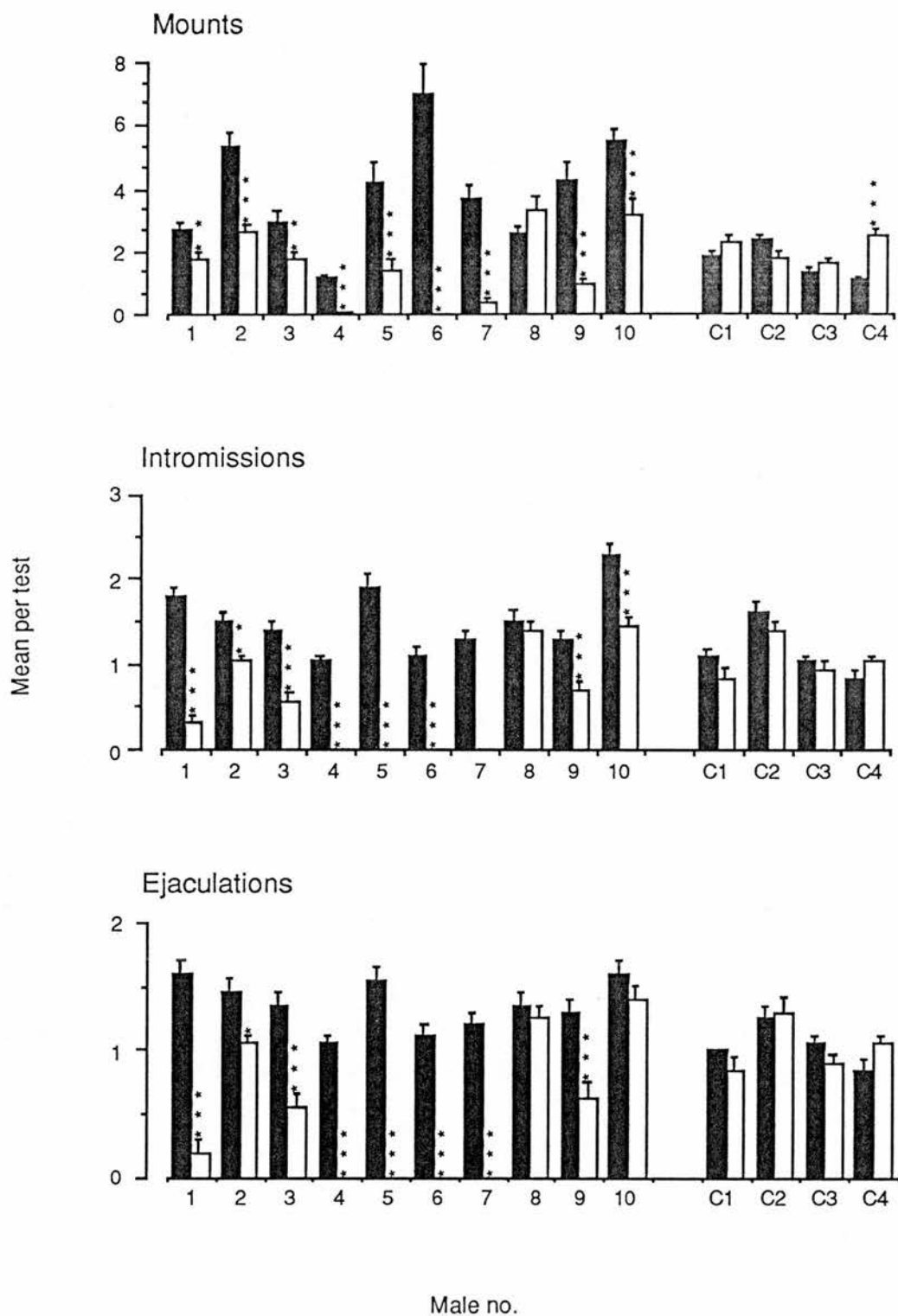


Fig. 4.5 Effects of lesions (males 1-10) or sham lesions (males C1-C4) on copulatory behaviour.

Histograms show mean \pm s.e.m. frequencies per test for each male pre- (solid bars) and post-lesion (open bars). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ Mann-Whitney U-test (within-animal comparisons).

No.	Mount Latency (secs.)		Intromission Latency (secs.)		Ejaculation Latency (secs.)	
	Pre	Post	Pre	Post	Pre	Post
1	4.65 +- 1.93	12.25 +- 6.35***	18.90 +- 4.17	ND	9.15 +- 0.93	ND
2	4.05 +- 0.39	5.30 +- 0.36***	13.00 +- 2.57	90.50 +- 65.02**	4.60 +- 0.20	4.40 +- 0.26
3	4.80 +- 0.60	54.16 +- 30.15***	10.90 +- 0.94	ND	5.70 +- 0.25	ND
5	2.20 +- 0.11	674.27 +- 140.31***	19.15 +- 14.15	ND	4.45 +- 0.97	ND
8	3.25 +- 0.54	3.60 +- 0.49	36.95 +- 20.00	34.45 +- 23.64	43.25 +- 38.21	4.55 +- 0.24
9	3.80 +- 0.25	53.92 +- 33.98***	15.70 +- 5.88	100.82 +- 55.09***	4.35 +- 0.11	5.70 +- 0.28***
10	2.65 +- 0.15	3.05 +- 0.40	7.30 +- 1.47	47.70 +- 37.29*	15.20 +- 7.36	4.80 +- 0.11
C1	13.10 +- 1.30	16.80 +- 1.74	29.35 +- 6.75	25.38 +- 2.51	11.85 +- 5.96	6.25 +- 1.99
C2	3.15 +- 0.27	72.40 +- 34.42	8.70 +- 0.79	154.00 +- 73.17***	18.00 +- 7.65	12.05 +- 7.41
C3	3.65 +- 0.25	3.10 +- 0.37	19.60 +- 4.30	11.56 +- 1.54	5.55 +- 0.54	5.67 +- 0.30
C4	3.80 +- 0.18	3.65 +- 0.28	18.18 +- 2.81	11.40 +- 1.16*	5.65 +- 0.17	5.95 +- 0.74

Fig. 4.6 Effects of lesions (males 1-10) or sham lesions (males C1-C4) on mount, intromission and ejaculation latencies.

Data are means +- s.e.m. for 20 pre-operative and 20 post-operative tests on each individual. Only those lesioned males that continued to mount post-operatively at sufficient frequencies for statistical analysis are shown here.

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ Mann-Whitney U-test (within-animal comparisons).

% of Total Mounts with:						
No.	Penile Erection		Pelvic Thrusting		Female Initiation	
	Pre	Post	Pre	Post	Pre	Post
1	100.00 +- 0.00	98.33 +- 1.62	89.58 +- 3.20	83.33 +- 5.50	35.00 +- 6.20	46.25 +- 9.14
2	100.00 +- 0.00	97.50 +- 2.44	54.70 +- 5.37	68.20 +- 6.07	50.10 +- 6.06	63.80 +- 8.65
3	94.66 +- 2.44	92.11 +- 5.60	74.48 +- 5.94	68.68 +- 9.83	56.46 +- 7.63	42.63 +- 10.14
5	91.61 +- 4.44	45.97 +- 12.87**	73.73 +- 6.21	0.00 +- 0.00***	47.90 +- 5.21	14.50 +- 5.92***
8	95.83 +- 2.86	100.00 +- 0.00	79.67 +- 5.43	79.86 +- 4.96	86.67 +- 4.80	79.17 +- 6.52
9	96.43 +- 3.48	100.00 +- 0.00	51.88 +- 6.50	87.18 +- 8.54**	71.82 +- 4.04	82.05 +- 10.01*
10	86.32 +- 4.65	87.58 +- 4.40	57.87 +- 4.60	74.52 +- 5.60*	47.17 +- 3.46	27.03 +- 5.92
C1	77.50 +- 5.17	62.92 +- 6.49	71.67 +- 5.90	49.56 +- 6.60*	84.17 +- 7.02	83.75 +- 5.22
C2	100.00 +- 0.00	100.00 +- 0.00	98.75 +- 1.22	95.42 +- 2.46	32.92 +- 6.78	27.50 +- 6.70
C3	100.00 +- 0.00	100.00 +- 0.00	96.67 +- 2.24	95.83 +- 2.86	10.00 +- 6.71	15.00 +- 6.22
C4	100.00 +- 0.00	98.33 +- 1.62	100.00 +- 0.00	83.08 +- 5.27*	67.50 +- 10.17	53.00 +- 7.72

Fig. 4.7 Effects of lesions (males 1-10) or sham lesions (males C1-C4) upon mount characteristics.

Data are means +- s.e.m. for 20 pre-operative and 20 post-operative tests on each individual. Only those lesioned males that continued to mount post-operatively at sufficient frequencies for statistical analysis are shown here.

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ Mann-Whitney U-test (within-animal comparisons).

There was no evidence that females showed any changes in sexual receptivity after their male partners were lesioned though significant decreases in the number of proceptive bouts per test shown by the female partners of 6 males (nos. 4, 5, 6, 7, 9 and 10) were seen following surgery - see Fig. 4.8. No similar decline was seen for the female partners of the 4 control males and in fact there was a significant increase in the case of the female partner of male no. C4 (this male was the one that also showed significant increases in all aspects of sexual arousal post-operatively).

Male	Pre-operative	Post-operative
1	1.05 +- 0.23	1.55 +- 0.40
2	5.45 +- 0.69	3.75 +- 0.45
3	2.75 +- 0.31	2.40 +- 0.38
4	5.30 +- 0.41	2.05 +- 0.28 **
5	3.45 +- 0.37	1.55 +- 0.28 **
6	4.10 +- 0.83	0.25 +- 0.12 **
7	1.95 +- 0.43	0.55 +- 0.17 *
8	5.05 +- 0.51	7.40 +- 0.62
9	9.90 +- 0.98	2.50 +- 0.31 **
10	3.55 +- 0.44	1.85 +- 0.40 *
C1	9.90 +- 1.03	9.25 +- 0.69
C2	1.10 +- 0.22	0.85 +- 0.20
C3	0.30 +- 0.21	0.40 +- 0.16
C4	1.05 +- 0.17	3.25 +- 0.43 **

Fig. 4.8 Effects on the proceptivity of female marmosets following hypothalamic lesioning of their male partners.

Data are means +- s.e.m. proceptive bouts per test for 20 pre-operative and 20 post-operative tests on each individual. * P<0.01, ** P<0.001 Mann-Whitney U-test (within-animal comparisons).

4.3.3 Effects on social behaviour

4.3.3.1 Grooming behaviour

The effects of these hypothalamic lesions upon frequencies of allogrooming and grooming invitations by both sexes are shown in Fig. 4.9. Although there are no consistent effects on grooming behaviour, in 9 out of 10 pairs, at least one aspect of this activity increased significantly post-operatively; only in the case of male no. 9 were there no increases in either male or female grooming behaviour. Although the results are not conclusive, there is an indication that - for male allogrooming and female grooming invitations at least - the greater the deficit in sexual behaviour, the greater the increases in grooming activity. This pattern is particularly clear in the cases in which post-operative mounting behaviour occurred at less than 50% of pre-operative rates (male nos. 2,4,5,6,7 and 9) - see Fig. 4.10. In these 6 cases, the percentage increase in both male allogrooming and females grooming invitations appear to be inversely related to sexual activity, and, in the case of the latter, Spearman's rank correlation reveals a significant result ($r_s = -0.886$, $n=6$, $P=0.05$). This pattern is not apparent for pairs in which sexual activity (i.e. mounting behaviour) continued at more than 50% of pre-operative levels (male nos. 1,3,8, and 10). No significant correlations were found between levels of sexual activity and male allogrooming or between sexual activity and female grooming invitations across the 20 pre-operative tests for the 14 pairs, suggesting that the pattern seen post-operatively in the case of some males is due to the decrease in sexual activity caused by the surgery.

No.	Male allogrooming		Female allogrooming		Male grooming invitations		Female grooming invitations	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
1	1.80 +- 0.26	3.50 +- 0.40 **	1.65 +- 0.48	2.05 +- 0.52	5.20 +- 0.89	6.90 +- 1.80	0.15 +- 0.11	1.45 +- 0.62
2	2.35 +- 0.67	0.80 +- 0.18	1.00 +- 0.27	0.45 +- 0.15	4.20 +- 0.69	1.15 +- 0.29 ***	2.30 +- 0.66	0.65 +- 0.26 ***
3	12.60 +- 1.00	15.40 +- 1.12	0.55 +- 0.37	1.30 +- 0.41	0.05 +- 0.05	3.30 +- 0.83 **	2.30 +- 0.65	6.20 +- 0.87 ***
4	3.15 +- 0.65	4.95 +- 0.63	0.30 +- 0.17	0.20 +- 0.11	0.25 +- 0.10	0.35 +- 0.16	0.75 +- 0.23	9.90 +- 1.06 ***
5	2.05 +- 0.41	3.45 +- 0.68	2.90 +- 0.51	1.45 +- 1.43 ***	3.70 +- 0.85	14.40 +- 1.11 ***	1.45 +- 0.49	4.15 +- 0.66 ***
6	5.00 +- 0.84	9.95 +- 1.05 ***	0.75 +- 0.30	0.45 +- 0.18	0.45 +- 0.17	1.10 +- 0.25	0.80 +- 0.26	7.45 +- 0.94 ***
7	6.75 +- 0.72	7.65 +- 0.74	0.40 +- 0.18	1.30 +- 0.45	2.60 +- 0.59	6.95 +- 1.28 **	2.50 +- 0.40	7.95 +- 0.77 ***
8	2.50 +- 0.40	0.30 +- 0.12 ***	12.95 +- 1.15	9.85 +- 0.69	12.95 +- 1.37	9.65 +- 0.80	0.90 +- 0.20	0.10 +- 0.07
9	3.90 +- 0.52	2.70 +- 0.69	0.05 +- 0.05	0.06 +- 0.06	0.50 +- 0.22	0.63 +- 0.25	4.35 +- 0.51	2.94 +- 0.66
10	6.90 +- 0.71	1.00 +- 0.25 ***	0.00 +- 0.00	2.05 +- 0.65 ***	2.00 +- 0.85	10.05 +- 2.17 ***	2.60 +- 0.57	0.60 +- 0.19 **
C1	9.35 +- 1.02	8.45 +- 0.80	0.00 +- 0.00	0.05 +- 0.05	0.00 +- 0.00	0.20 +- 0.19	0.60 +- 0.23	0.95 +- 0.22
C2	1.05 +- 0.29	0.80 +- 0.27	0.00 +- 0.00	0.00 +- 0.00	0.00 +- 0.00	0.05 +- 0.05	0.10 +- 0.07	0.35 +- 0.20
C3	6.15 +- 0.61	2.80 +- 0.41 ***	0.00 +- 0.00	0.00 +- 0.00	0.00 +- 0.00	0.05 +- 0.05	0.55 +- 0.25	0.05 +- 0.05
C4	6.10 +- 1.40	2.40 +- 0.47 **	0.00 +- 0.00	0.00 +- 0.00	0.05 +- 0.05	0.00 +- 0.00	0.55 +- 0.21	0.45 +- 0.11

Fig. 4.9 Effects of lesions (males 1-10) or sham lesions (males C1-C4) on allogrooming and grooming invitations.

Data are means +- s.e.m. for 20 pre-operative and 20 post-operative tests on each individual.

* P<0.05, ** P<0.01, *** P<0.001 Mann-Whitney U-test (within-animal comparisons).

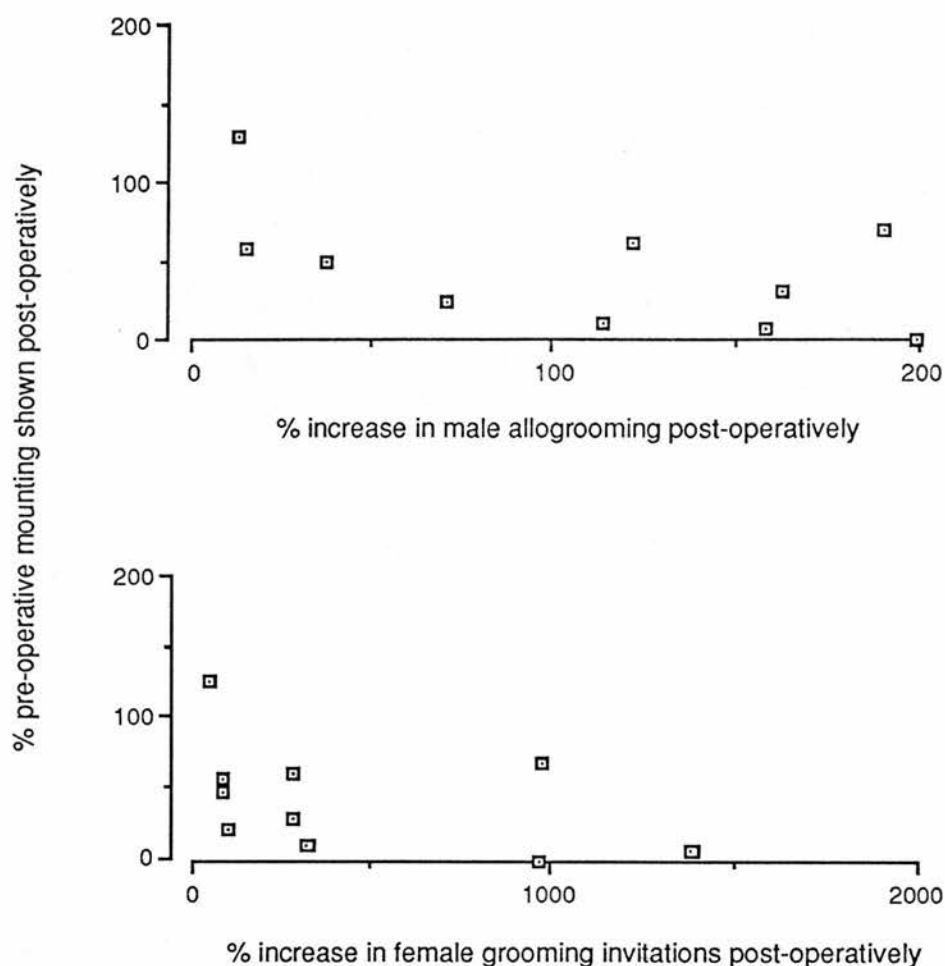


Fig. 4.10 Correlation between % decrease in mounting behaviour and % increase in grooming activity.

See text for details of the statistical correlation between the decrease in mounting activity and the increases in grooming behaviour seen post-operatively.

4.3.3.2 Scent marking and genital presents

There were no clear effects on either scent marking or genital presents in these males. Indeed, these behaviours were highly variable between and within males (see Fig. 4.11), and there were significant changes in both behaviours within the control males which makes analysis of the data

impossible.

No.	Scent marking		Genital presents	
	Pre	Post	Pre	Post
1	0.00 +- 0.00	0.70 +- 0.37	0.00 +- 0.00	0.00 +- 0.00
2	1.00 +- 0.35	5.75 +- 1.17 ***	0.00 +- 0.00	0.20 +- 0.09
3	6.00 +- 0.98	1.20 +- 0.43 ***	2.90 +- 0.61	0.00 +- 0.00 ***
4	5.00 +- 4.87	0.35 +- 0.16	0.00 +- 0.00	0.00 +- 0.00
5	9.80 +- 1.08	6.85 +- 0.66	2.90 +- 0.63	0.05 +- 0.05 ***
6	0.80 +- 0.31	1.20 +- 0.37	0.70 +- 0.21	0.50 +- 0.36
7	17.55 +- 1.22	4.00 +- 0.73 ***	8.15 +- 1.32	8.15 +- 1.32
8	2.70 +- 0.47	4.55 +- 0.63	2.50 +- 0.49	0.20 +- 0.09 ***
9	8.50 +- 1.07	2.38 +- 0.61 ***	0.00 +- 0.00	0.00 +- 0.00
10	29.05 +- 2.71	26.75 +- 2.66	1.35 +- 0.33	4.30 +- 0.56 ***
C1	2.80 +- 0.45	4.90 +- 1.39	0.00 +- 0.00	2.15 +- 0.69 **
C2	9.80 +- 1.63	28.30 +- 2.69 ***	0.10 +- 0.10	0.15 +- 0.08
C3	1.65 +- 0.82	8.25 +- 1.59 ***	0.00 +- 0.00	0.00 +- 0.00
C4	48.60 +- 3.07	26.40 +- 3.22 ***	58.00 +- 3.67	16.80 +- 3.03 ***

Fig. 4.11 Effects of lesions (males 1-10) or sham lesions (males C1-C4) on scent marking and genital presents.

Data are means +- s.e.m. for 20 pre-operative and 20 post-operative tests on each individual. ** P<0.01, *** P< 0.001 Mann-Whitney U-test (within-animal comparisons).

4.3.4 Effects of oestrogen administration to the females

During oestradiol treatment, a Wilcoxon test between animals reveals that the females showed a significant increase in proceptive bouts (from mean +- s.e.m. 1.7 +- 0.69 to 7.7 +-1.47 per test, T=0, n=11, P<0.001) - see Fig. 4.12.

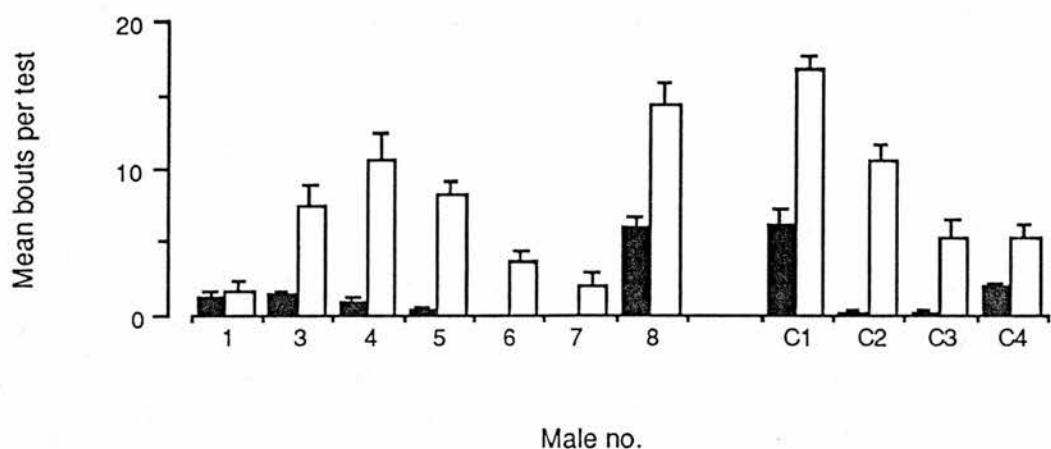


Fig. 4.12 Effects of administration of oestrogen on the proceptive behaviour of females paired with lesioned (nos. 1-8) or sham lesioned (nos. C1-C4) males.

Data are mean \pm s.e.m. for 5 tests for each pair before (solid bars) and during oestrogen treatment (open bars). Due to the small number of tests, no statistical analysis was undertaken.

However, this did not induce a recovery in the sexual behaviour of the 7 lesioned males studied. The number of mounts per test were not significantly increased (from 1.26 ± 0.61 to 1.34 ± 0.49 per test), nor were male pre-copulatory tongue flicking bouts (from 0.8 ± 0.41 to 2.14 ± 1.12 per test), anogenital investigation of the female (from 1.37 ± 0.53 to 0.74 ± 0.27 per test) or approaches of the male to the female (from 7.17 ± 1.92 to 7.66 ± 1.89 per test) as evidenced by Wilcoxon tests between animals. When the sham lesioned males are considered, in 3 cases there was an increase in mount frequency (50% increase for C2, 156% for C3 and 11% for C4), however C1 showed no rise in mounting frequency in response to a significant increase in proceptive bouts from the female. There was a consistent rise in tongue flicking bouts for the control males (135.3% increase for C1, 233.3% for C2, 500% for C3 and 54.5% for C4). However, there was no consistent effect of greater proceptivity among the females on anogenital investigation of the female (125% increase for C1, 33.3%

increase for C2, 0% increase for C3, 26.1% decrease for C4) or on approaches of the male to the female (36.3% decrease for C1, 15.6% decrease for C2, 86.4% increase for C3, 11.3% increase for C4). This lack of consistent effect of increased female proceptivity on the sham lesioned males' behaviour may, however, be because the males were behaving optimally in the tests before oestrogen treatment.

4.3.5 Effects on agonistic encounters with a strange male

The effects of these lesions on agonistic behaviour are shown in Fig. 4.13. Behavioural patterns were divided into three categories: physical aggression (attacks involving hitting and biting, lunges and chases), threats (vocal threats, arch ruffles and genital presents) and submissive behaviours (crouches, grimaces, tongue flicking, squeals and flees). This classification is based upon the apparent functional significance of the behaviours during agonistic encounters, and all behaviours within each category are not assumed to be controlled by the same neural mechanisms. In every test where agonistic interactions occurred, resident males (i.e. males in the lesion or sham lesion groups) became dominant over intruders. Five out of six lesioned males which exhibited physical aggression pre-operatively, showed a decrease in such behaviour during the post-operative test. However, sham lesioned males also showed a similar decrease in physical aggression. The effects of these lesions upon threat behaviour were more consistent, and a small but statistically significant decrease in threats occurred during post-operative tests as evidenced by a Wilcoxon test between animals ($T=1.5$, $N=7$, $P<0.05$) whereas there were no consistent changes in this behaviour in the control pairs (Fig. 4.13).

		% of 30 second blocks with 'resident' showing:							
		Attack latency (secs)		Physical aggression		Threats		Submissive behaviour	
No.		Pre	Post	Pre	Post	Pre	Post	Pre	Post
2		75	58	20.0 (3)	6.7 (30)	96.7 (30)	100.0 (30)	0.0 (30)	0.0 (30)
3		ND	ND	0.0 (30)	0.0 (30)	0.0 (30)	0.0 (30)	0.0 (30)	0.0 (30)
4		ND	ND	0.0 (30)	0.0 (30)	40.0 (30)	3.3 (30)	0.0 (30)	0.0 (30)
5		2	ND	16.7 (30)	0.0 (30)	90.0 (30)	20.0 (30)	0.0 (30)	0.0 (30)
6		ND	ND	6.7 (30)	0.0 (30)	36.7 (30)	0.0 (30)	0.0 (30)	0.0 (30)
7		455	ND	60.0 (30)	0.0 (30)	80.0 (30)	60.0 (30)	6.7 (30)	0.0 (30)
8		12	147	100.0 (2)	23.3 (30)	100.0 (2)	96.7 (30)	0.0 (2)	0.0 (30)
10		75	3	42.9 (7)	80.0 (10)	100.0 (7)	80.0 (10)	0.0 (7)	1.0 (10)
		123.8 +- 84.2	245.0 +- 175.0	30.8 +- 12.4	15.7 +- 11.2	67.9 +- 13.3	50.9 +- 16.6 *	0.8 +- 0.8	0.1 +- 0.1
C1		100	800	60.0 (30)	16.7 (30)	70.0 (30)	86.7 (30)	23.3 (30)	3.3 (30)
C2		420	ND	3.3 (30)	0.0 (30)	43.3 (30)	30.0 (30)	3.3 (30)	0.0 (30)
C3		20	88	100.0 (3)	83.3 (7)	100.0 (3)	100.0 (7)	30.0 (3)	0.0 (7)
C4		56	103	58.3 (12)	10.0 (30)	83.3 (12)	100.0 (30)	0.0 (12)	3.3 (30)
		149.0 +- 91.8	330.3 +- 234.9	55.4 +- 19.9	27.5 +- 18.9	74.2 +- 11.2	79.2 +- 16.7	14.2 +- 7.4	1.65 +- 1.0

Fig. 4.13 Effects of lesions (males 1-10) or sham lesions (males C1-C4) on frequencies of aggressive and submissive behaviour shown by residents towards a male intruder.
 ND: no attacks occurred. Total numbers of 30 second intervals are given in parentheses.
 * P<0.05 Wilcoxon test (between-animal comparison).

Physical aggression or threat behaviour was rarely seen from intruder males, whereas submissive patterns were scored during $80.8 \pm 8.8\%$ of 30 second intervals during pre-operative tests. A significant decline in submissive behaviours of intruders occurred when their partners had received lesions as evidenced by a Wilcoxon test between animals (post-operative mean \pm s.e.m. $44.6 \pm 16.1\%$ of 30 second intervals; $T=0$, $n=7$, $P<0.001$).

4.3.6 Effects on plasma testosterone levels and androgen-dependent morphology

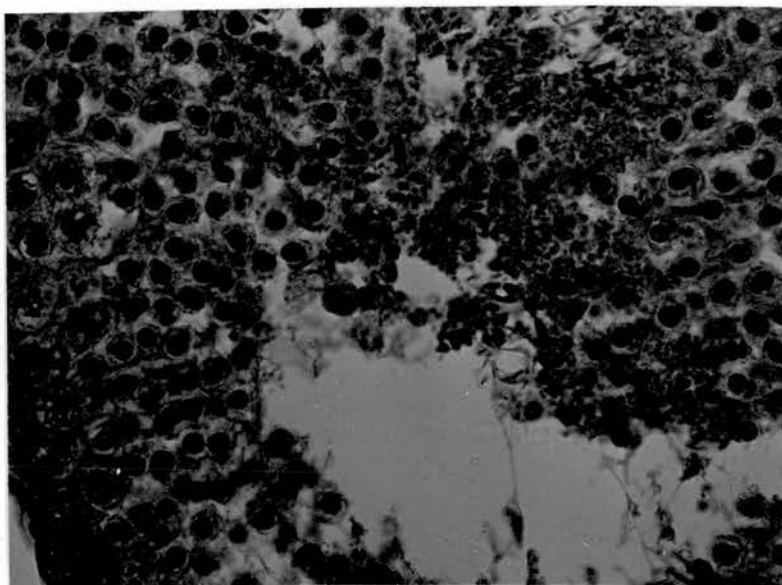
For the 10 males, pre-operative plasma testosterone levels ranged from 1.4 - 83.7 ng/ml (mean 27.22 ng/ml) as compared to post-operative levels of 1.0 - 40.2 ng/ml (mean 15.57 ng/ml). Male no. 7 was the only case in which a statistically significant decrease in plasma testosterone occurred during the post-operative tests (pre-lesion 5.8 - 59.4 ng/ml, mean 22.23, post-lesion 1.0 - 16.4 ng/ml, mean 6.41, $U=10$, $n=9$, $P<0.05$) - see Fig. 4.14, and there was no significant decline across the 10 lesioned males as evidenced by a Wilcoxon test. Male no. 7 was therefore treated with testosterone propionate by daily intramuscular injection (0.75 mg for 24 days). This treatment restored plasma testosterone to 7.2 - 32.0 ng/ml (mean 18.2 ng/ml) but no significant change in any measurement of sexual behaviour occurred during 10 tests conducted under these conditions, i.e. no anticipatory erections, attempted mounts or mounts were seen during these 10 tests, and the male showed only 1 bout of precopulatory tongue flicking - compared with a mean frequency of 1.9 bouts per test during the 20 pre-operative tests.

Male	Pre-operative	Post-operative
1	20.26 +- 6.53	12.08 +- 3.87
2	13.93 +- 6.52	21.87 +- 4.05
3	49.15 +- 6.22	23.51 +- 7.09
4	29.28 +- 1.93	28.07 +- 3.45
5	34.23 +- 5.72	37.90 +- 3.27
6	17.47 +- 5.31	6.42 +- 3.02
7	22.22 +- 5.82	6.41 +- 1.80 *
8	16.78 +- 6.24	15.61 +- 3.62
9	42.16 +- 2.75	39.70 +- 4.12
10	15.89 +- 3.64	16.43 +- 5.89
C1	14.84 +- 2.50	15.06 +- 2.10
C2	36.27 +- 5.62	40.29 +- 7.42
C3	29.84 +- 4.77	28.83 +- 5.61
C4	34.95 +- 1.89	30.59 +- 4.71

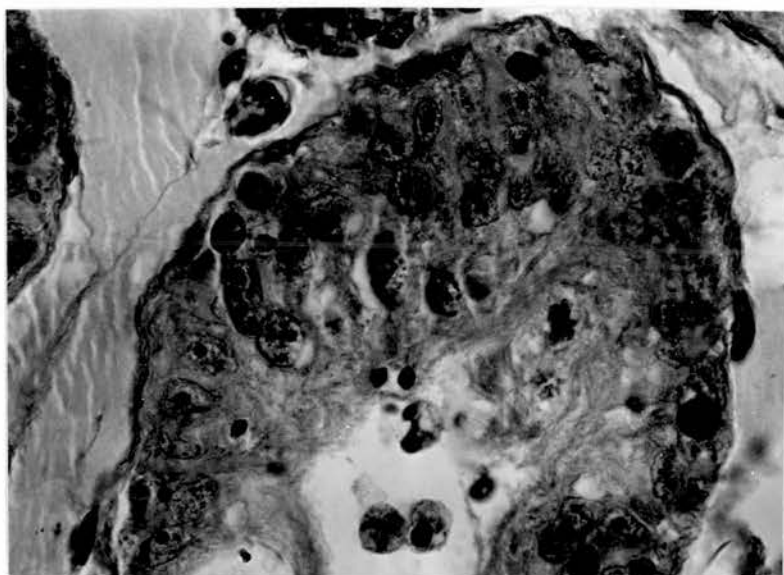
Fig. 4.14 Effects of lesions (males 1-10) or sham lesions (males C1-C4) on plasma testosterone levels.

Mean +- s.e.m. for weekly pre- and post-operative blood samples. Data are ng/ml. *P<0.05 Mann-Whitney U-test (within-animal comparisons).

The reproductive tracts of the lesioned males were grossly normal, and androgen-dependent penile spines were visible on the glans penis. Spermatogenesis was unaffected (see Fig. 4.15), except in the case of male no. 9 in which the seminiferous tubules contained no spermatozoa and the epithelium had undergone degeneration (see Fig. 4.15). Male no. 9 was the only individual which exhibited signs of ill-health (see below) after the POA had been lesioned. Despite this, plasma testosterone levels remained within the normal range and did not differ significantly from measurements made before surgery.



Male no. 6



Male no. 9

Fig. 4.15 Representative sections from the testes of male nos. 6 and 9.

4.3.7 Effects on general health

In all but one case (male no. 9), the males recovered rapidly from surgery and remained in good health until the conclusion of the study. There was a tendency towards hyperphagy in the first 2-3 weeks post-operatively and most of the males gained weight during the post-operative period. However, in most cases, this weight gain was minimal (average - 10.8% of pre-operative weight) and was comparable with that of the 4 control males (see Fig. 4.16). Interestingly, the largest effects were seen in the males with most damage to the VmH (male nos. 1, 2 and 3).

Male	Body Weight		Gain	Male	Body Weight		Gain
No.	Pre	Post	(%)	No.	Pre	Post	(%)
1	300	389	29.7	8	324	380	14.7
2	329	410	24.6	9	307	276	-11.2
3	329	402	22.2	10	342	351	2.6
4	350	367	4.9	C1	363	388	6.9
5	347	356	2.6	C2	334	364	9.0
6	320	347	8.4	C3	343	357	4.1
7	334	332	-3.6	C4	398	441	10.8

Fig. 4.16 Pre- and post-operative body weights (g) and % weight gain for lesioned males 1-10 and control males C1-C4.

The body temperatures of the lesioned males were checked during the post-operative period and, in all but one case (male no. 9), the temperatures fell within the normal range (101-103 °F for 8 normal males).

Male no. 9 experienced difficulties in the immediate post-operative period and hand feeding was necessary for 3-4 weeks to maintain body weight. After this initial period, the male's general health improved and he was reintroduced into the study. However, after 16 post-operative tests, the health of this male again deteriorated and subsequent tests were

abandoned. At the time of euthanaesia, this male had lost 11.2% of his pre-operative body weight and his basal body temperature was abnormally low (95 °F).

4.5 Discussion

Before commencement of this study, a substantial number of pilot animals were lesioned in order to calculate correction factors for the atlas co-ordinates (as described in Chapter 3) and to maximise the accuracy of the surgery. It was therefore assumed, following the successful completion of this preliminary work, that the males could be lesioned in such a way as to create groups upon which an analysis of variance would be possible. Unfortunately, the stereotaxy was not as accurate as expected, leading to a scatter of lesion placement across the 10 males. Analysis of variance was therefore impossible, so that the only inference as to the effect of lesion placement is that gained by analysis of the data for each male individually.

Although this fact limits the conclusions that can be drawn from this data, many of the effects seen in this study are worthy of discussion.

Hypothalamic lesions affected both sexual arousal and copulatory behaviour in these male marmosets. The most profound deficits in arousal and copulation followed thermal lesions placed under the anterior commissure at the level of the dorsalis anterior and paraventricular nuclei, at the junction between the anterior hypothalamus and the preoptic area (POA-AH). This is in agreement with the only other study that has been carried out on male primates, in which lesions of the MPOA-AH of rhesus monkeys caused severe deficits in sexual activity (Slimp et al., 1978).

Of particular interest is the fact that, in this study, the males that showed the

most profound deficits in copulatory behaviour also exhibited dramatic decreases in sexual arousal - as evidenced by anticipatory erections, anogenital investigations of the female and precopulatory tongue flicking. This finding is in conflict with many other studies in which it has been found that lesions within the MPOA-AH spare aspects of sexual arousal in cats (Hart, 1980), goats (Hart, 1986), mice (Bean et al., 1981) and hamsters (Powers et al., 1987).

It would appear that, in the common marmoset, the POA-AH provides a link between sexual arousal and copulatory activity. Of interest here is the case of male no. 1 who received a bilateral thermal lesion at the level of the VmH. This male continued to mount regularly but appeared to experience difficulty in intromitting. This male's post-operative sexual behaviour resembled that of intact males after penile desensitization (Dixson, 1986). It is possible that the medial hypothalamus plays an additional role in copulatory behaviour in primates though further work employing more selective lesioning techniques within the medial hypothalamus is required.

Another case of interest is that of male no. 8 whose lesion was confined to the medial and lateral nuclei of the POA. No changes in any aspect of sexual arousal or copulatory behaviour were seen post-operatively, arguing against a role for the POA in masculine sexual arousal. However, in male no. 9 in which the lesion was larger (3.2mm^3 compared to 2.41mm^3 for male no. 8) and damaged the POA more extensively, profound deficits in both sexual arousal and copulatory behaviour were seen. This example illustrates the importance of lesion volume in the interpretation of data though the fact that male no. 9 was ill during the post-operative period must also be borne in mind in this instance.

In summary, lesions within the POA and the POA-AH of male common marmosets profoundly affect sexual arousal and copulatory activity if a

relatively large portion of the structures are destroyed. The results suggest that these structures or fibres of passage passing through this area are involved in the control of both aspects of sexual behaviour as the sparing of components of sexual arousal seen in other studies on a number of mammalian species was not evident in this study. More rostral or caudal lesions have less profound behavioural effects, and it is therefore suggested that the POA-AH provides a link between arousal mechanisms and the control of copulatory behaviour. The latter control systems are thought to be situated in more caudal hypothalamic areas (Yoshimatsu, 1983; Oomura et al., 1984).

The effects of these lesions on social behaviours are not as clear. There does appear to be an inverse relationship between sexual behaviour and grooming activity as the pairs that experienced the least sexual behaviour post-operatively also showed dramatic increases in grooming behaviour. In pair tests on rats following thermal lesions within the MPOA which inhibited sexual activity, displacement behaviours of drinking and hindlimb scratching emerged (Hansen & Drake af Hagelsrum, 1984). It was found that the amount of displacement activity was inversely related to the strength of masculine sexual behaviour, and the authors concluded that these behaviours emerged due to the thwarting of sexual motivation by these lesions. In the present study, eating and drinking behaviours were precluded by the removal of food trays and water bottles before commencement of each test, and scratching behaviour was not scored. However, it is possible that the increases in allogrooming and grooming invitations by the males and females of pairs that experienced decreases in sexual behaviour may be examples of displacement activities. For a displacement activity to emerge, a thwarted motivation must be present. In the case of the rat study, this was the continuation of sexual arousal

following MPOA lesions which inhibit copulatory behaviour. However, in the present study, it is argued that the male marmosets are less sexually arousable following these lesions but it is possible that a second motivation is thwarted by these lesions, and that is the urge to strengthen the pair bond. Marmosets are generally monogamous and sexual behaviour serves to strengthen the bond between males and females, particularly around the time of parturition (Evans & Poole, 1984). In these pair tests, sexual behaviour may serve the same purpose, and it is this lack of a reinforcing interaction between the pairs that results in the emergence of displacement activities. If this is so, the displacement activities displayed may also serve as a positive reinforcing interaction between the partners that, to a certain extent, mirrors the effects of copulation.

A similar effect on grooming behaviour has been seen between pairs of marmosets following lesions placed in the anterior hypothalamus of the female which lead to a reduction in proceptivity (Kendrick & Dixson, 1986). However, treatment of talapoin monkeys with naloxone, which inhibits the opiate system, also results in increases in grooming activity (Fabre-Nys et al., 1982) and it may be that these same systems have been disrupted in the present study.

There is evidence to implicate the MPOA in the control of scent marking behaviour in the male gerbil (Yahr et al., 1982; Commins & Yahr, 1984; Yahr & Ulibarri, 1987) and to indicate that the central actions of androgens within the MPOA are important (Thiessen et al., 1973; Yahr et al., 1982). Unfortunately, in the present study, the effects on scent marking were inconsistent and the control males also exhibited significant alterations in this behaviour, thus precluding any meaningful discussion of the data. Indeed, of the two males that received lesions centred within the MPOA, one showed a slight increase in scent marking behaviour post-operatively (male

no. 8) and the other showed a significant decline (male no. 9).

The results following oestrogen treatment of the females are interesting on two counts. Firstly, despite the fact that some of the females had encountered no (or very little) sexual interest from their male partners in the 25 tests preceding oestrogen treatment, they still exhibited a significant increase in proceptive bouts during the 5 post-oestrogen tests. This illustrates the very powerful effect of oestrogen on proceptive behaviour even under circumstances when a sexual response from the gonadally intact male partner is extremely unlikely. There is individual variation between females with respect to the effects of oestrogen on proceptive behaviour, and it has been found that those females that exhibit the highest levels of proceptivity without oestrogen stimulation will respond maximally to this treatment (Dixon & Lloyd, unpublished data). The response of the male towards the female also influences the levels of proceptive behaviour, as 87.7% of all female tongue flicking bouts occurred during eye contact with the male. It would appear that the immobile behaviour exhibited by female marmosets will occur without any cue from the male, but eye contact appears to be a necessary prerequisite to tongue flicking in the majority of cases (Dixon & Lloyd, unpublished data). In many of the tests following oestrogen treatment, the lesioned males appeared to be actively avoiding eye contact with their female partners. This 'eye-contact proceptivity' has been noted in a number of primate species including the chacma baboon (Bielert, 1986), the gorilla (Hess, 1973) and the talapoin (Dixon et al., 1975).

The second point of interest is that, despite the increased stimulus of an oestrogen-treated, and therefore proceptive and presumably attractive, female, no increase in sexual interest or copulatory behaviour was apparent in the lesioned males, though there was a marked increase in precopulatory behaviours and copulatory activity in the sham lesioned controls. This result

lends further weight to the argument that the male marmosets receiving thermal lesions within the POA-AH experience deficits in sexual arousal as well as in copulatory behaviour. If the males were still sexually interested in the females, one would expect to see increases in approaches, tongue flicking or mount attempts (hip touches) and this clearly did not happen. Of note here is the case of male no. 4, who exhibited aggression towards his female partner in response to a number of her proceptive displays and was clearly irritated by her persistent sexual invitations.

From the preliminary data on agonistic behaviour, it would appear that there is a decline in aggression in these males post-operatively paralleled by a decline in the level of submissive behaviour expressed by the intruder. It seems unlikely that this decrease in physical aggression and threat behaviour is due solely to recognition of the intruder and memory of the previous encounter, as this would presumably result in at least the same level of submissive gestures from the intruder during the second test, in an attempt to discourage aggressive behaviour from the resident. It is interesting that the clearest decreases in aggressive behaviour were seen in those males that experienced the most profound deficits in sexual behaviour. The LPOA (Roberts et al., 1967) and its lateral connections with the MFB (Bergquist, 1970) have been implicated in the control of aggressive behaviour in the opossum, but those researchers that have examined effects of MPOA-AH lesions on aggressive behaviour have reported negative results. For example, the dominance-subordinance relationships between male dogs are unaltered by these lesions (Hart, 1974). However, in the present study, in the post-operative tests where a hierarchical relationship was still apparent, the resident was still the dominant animal indicating similar results to those of Hart; it was only the absolute levels of aggression performed that were decreased. The VmH appears particularly important in

the control of aggression, and threat behaviour has been elicited from this area in the marmoset (Lipp & Hunsperger, 1978). Unfortunately, the only male in this study that received a lesion within this neural area was not subjected to an aggression test.

It is interesting that tongue flicking was performed by the intruders in response to vocal threats or physical aggression from the resident male. In this context the behaviour was clearly a submissive gesture and not a sexual invitation, and the use of such proceptive invitations as submissive postures in other primate species has been described (Hausfater & Takacs, 1987) and recently the use of tongue flicking during agonistic encounters between cottontop tamarins has been reported (Epple et al., 1987) though, in this instance, it was interpreted as a dominance display. It is possible that the high levels of tongue flicking seen between some of the pairs during the pre-operative tests may have been partly submissive in nature to discourage aggression, rather than to encourage sexual behaviour, and this may account for the gradual declines over a number of tests as the animals became more familiar with each other.

The effects of the thermal hypothalamic lesions are not due to effects on the hypothalamic-pituitary axis as plasma testosterone levels, spermatogenesis, and androgen-dependent morphology remained normal in all but two cases, and the decline in plasma testosterone levels in one of these individuals was proved not to be the cause of the behavioural deficit. There was a high variability in the plasma levels of testosterone within animals across the samples. This was minimized by collecting all the samples at the same time of the day but, in retrospect, more meaningful data could have resulted from a more frequent bleeding regime, thus increasing the numbers of pre- and post-operative samples.

The effects were also not due to influences on erectile mechanisms, as has

been previously suggested (Pfaff, 1980), as all the lesioned males showed a full penile erection on at least one post-operative test.

Finally, the general health of the males was good in all but one case, indicating that the behavioural effects seen cannot be attributed to a nonspecific effect on the animal's physiological wellbeing.

CHAPTER 5

EXPERIMENT 2: OXYTOCIN, β -ENDORPHIN AND MASCULINE SEXUAL AND SOCIAL BEHAVIOUR IN A PAIR TEST SITUATION

5.1 Introduction

It is clear from the results given in Chapter 4 that the POA-AH is vitally important in the control of masculine sexual behaviour in the common marmoset though the technique used so far i.e. thermal lesions, causes damage to cell bodies, axons, fibres of passage and terminals of neurons whose cell bodies are outside the hypothalamus. It is therefore now important to elucidate more precisely the components within the POA-AH that are involved in this control mechanism. This area contains a large number of neurotransmitter systems, many of which have been implicated in the control of copulation and/or penile erections (following either peripheral or central administration) in a number of mammalian species - see Chapter 2. In an attempt to elucidate the role of two neuroactive peptides within the POA-AH, bilateral intracerebral cannulations were performed on a number of males.

This experiment was carried out in two parts, the first was an attempt to measure plasma oxytocin levels in male marmosets following ejaculation and in female marmosets during vaginocervical stimulation, which has been found to result in increases plasma levels in a number of other mammalian species - see Chapter 2. The second part of the study was to administer oxytocin and β -endorphin via bilateral indwelling cannulae into the POA-AH to study the effects on sexual and social behaviour and erectile responses in males. β -endorphin was chosen as this peptide has been found to inhibit the sexual behaviour of male rats following bilateral infusion into the MPOA-AH (Hughes et al., 1987).

5.2 Materials and Methods

5.2.1 Animals

9 males and 9 females were selected using the criteria outlined in Chapter 3 for animals used in pair tests. The females were ovariectomized and received hormone treatment during part of the study as described below.

5.2.2 Protocol for plasma oxytocin measurement

5.2.2.1 Male testing regime and blood sampling

6 males were pair tested with ovariectomized females and the male removed from the cage immediately after ejaculation. Blood samples (1ml) were drawn from the femoral veins using heparinized syringes chilled in ice. All samples were collected within 90 seconds after the occurrence of ejaculation and centrifuged at 4,500rpm in a chilled centrifuge for 5 minutes. The plasma was then frozen in dry ice and stored at -20°C until assayed for oxytocin (see Chapter 3). One experimental bleed and one control bleed (no pair test) was taken from each male and the order of the bleeds was randomized across the 6 males.

5.2.2.2 Female testing regime and blood sampling

9 females were implanted subcutaneously with 2 silastic implants of oestradiol (see Chapter 3) and 5 days later an experimental blood sample was collected from one group (n=4) and control blood sample from the other group (n=5). The experimental bleed consisted of cervical stimulation by means of a plastic rod inserted 2.5cms. into the vagina and moved gently up and down. The blood sample was started 10 seconds after commencement

of the stimulation and this stimulation continued until the full sample (1.0ml) had been collected. A control bleed was carried out in the same way but no stimulation was applied. The samples were centrifuged at 4,500rpm for 5 minutes in a chilled centrifuge and the plasma immediately frozen in dry ice. The samples were stored at -20°C until assayed for oxytocin (see Chapter 3). The implants were then removed and the females left for 2 weeks. After this time the females were reimplanted and 5 days later they were bled again, with the second group now receiving the experimental bleed and the first group the control bleed. The samples were treated in the same way.

5.2.3 Surgery (Cannulations)

Cannulae were implanted bilaterally into the hypothalamus in 9 males, using the surgical procedures outlined in Chapter 3. The stereotaxic co-ordinates were calculated to place the cannulae tips within the MPOA-AH. In all cases, recovery was rapid, though the males were not pair tested for at least 1 month following surgery to ensure that the incision sites had fully healed.

5.2.4 Intracerebral infusion technique

Infusions of β -endorphin, oxytocin, physiological saline and artificial CSF were made prior to each pair test as described in Chapter 3. 8 males received 2 tests following an infusion of 50ng oxytocin, 2 following 100ng oxytocin and 2 following physiological saline. The order of these infusions was randomised across the 8 males.

In the second part of the study, 5 males were given 2 infusions of 40 pmols β -endorphin, 2 infusions of 80 pmols β -endorphin, and 4 infusions of artificial CSF, all followed by a pair test. The order of these infusions was randomised across the 5 males.

5.2.5 Behaviours scored

The specific behaviours listed here are defined in detail in Chapter 3.

During the pair tests the following behaviours were scored for the male; anticipatory penile erection, number and total duration of erections, precopulatory tongue flicks, anogenital investigation of the female, mount attempts, mounts, mount latency, intromissions, intromission latency, ejaculations, ejaculation latency, whether the mount included an erection and/or pelvic thrusting, self-anogenital investigation, allogrooming, grooming invitations, scent marking, genital presents and aggression. For the female the following were scored; proceptive bouts, mount refusals, mount terminations, whether or not the mount was initiated by her, allogrooming, grooming invitations, and aggression.

5.2.6 Histology

At the termination of the study, the brains were prepared and treated in the manner described for staining with luxol fast blue and cresyl fast violet in Chapter 3, though, in this case, the tissue was sectioned at 60µm.

5.3 Results

5.3.1 Plasma oxytocin following ejaculation and during vaginocervical stimulation

The results from both the males and the females were not clear. In all but 3 samples, levels of oxytocin were below 3.0pg/ml (the detection limit of the assay) and therefore unmeasurable. In addition, the samples that were detectable were all control bleeds:- 1 male (25.0 pg/ml) and 2 females (19.0 and 4.0 pg/ml respectively).

5.3.2 Histology

Fig. 5.1 shows the positions of the cannulae relative to the various hypothalamic nuclei in each male as well as the approximate AP position of the cannulae tips.

	OTHER			POA				AH				OTHER			AP AT TIP
	DB	IC	FDB	PM	APL	APM	APP	DAH	ALH	AAH	PvH	ADH	Vm	Dm	
1								*	*	*	*	*	*	*	9.3
2								*	*	*	*	*	*	*	9.4
3				*	*	*	*	*	*	*	*				9.7
4				*	*	*	*	*	*	*	*				9.8
5				*	*	*	*	*	*	*	*				9.8
6			*	*	*	*	*	*							10.0
7			*	*	*	*	*								10.3
8			*	*	*	*	*								10.3
9			*	*	*	*	*								10.4

Fig. 5.1 Positions of hypothalamic cannulae and the adjacent nuclei in male marmosets (nos. 1-9).

The position of the cannulae tips are also shown in mm anterior to interaural zero (AP). Adjacent nuclei = within 1mm³ of cannula tip.

- DB = diagonalis Brocae

FDB = fasciculus diagonalis Brocae

APL = lateral preoptic nucleus

APP = preoptic periventricularis nucleus

AAH = anterior hypothalamic area

PvH = paraventricular nucleus

Vm = ventromedial nucleus
- IC = insulae Callejae

PM = nucleus preopticus medianus

APM = medial preoptic area

DAH = dorsalis anterior nucleus

ALH = lateral hypothalamic area

ADH = dorsal hypothalamic area

Dm = dorsomedial nucleus

Here, and subsequently, the males are numbered 1-9 in order of cannula placement relative to interaural zero i.e. male no. 1 has the most caudal and male no. 9 the most rostral cannulae. From the position of the cannulae the males were divided in 3 groups:- group A (male nos. 1 and 2) had cannulae

within the antero-medial hypothalamus, group B (male nos. 3, 4 and 5) had cannulae within the anterior hypothalamus under the anterior commissure, and group C (male nos. 6, 7, 8 and 9), in which the cannulae were situated rostral to the anterior commissure in the POA. Fig. 5.2 shows a photograph of a representative section from one of the males (no. 6) showing the cannulae tracts and Figs. 5.3 and 5.4a-c show schematic representations of these cannulae placements. Within each group there was very little variation in AP, L or V co-ordinates between individuals so that the members of each group are considered to be homogeneous.



Fig. 5.2 Photograph of a representative section from male no. 6 showing the cannulae tracts.

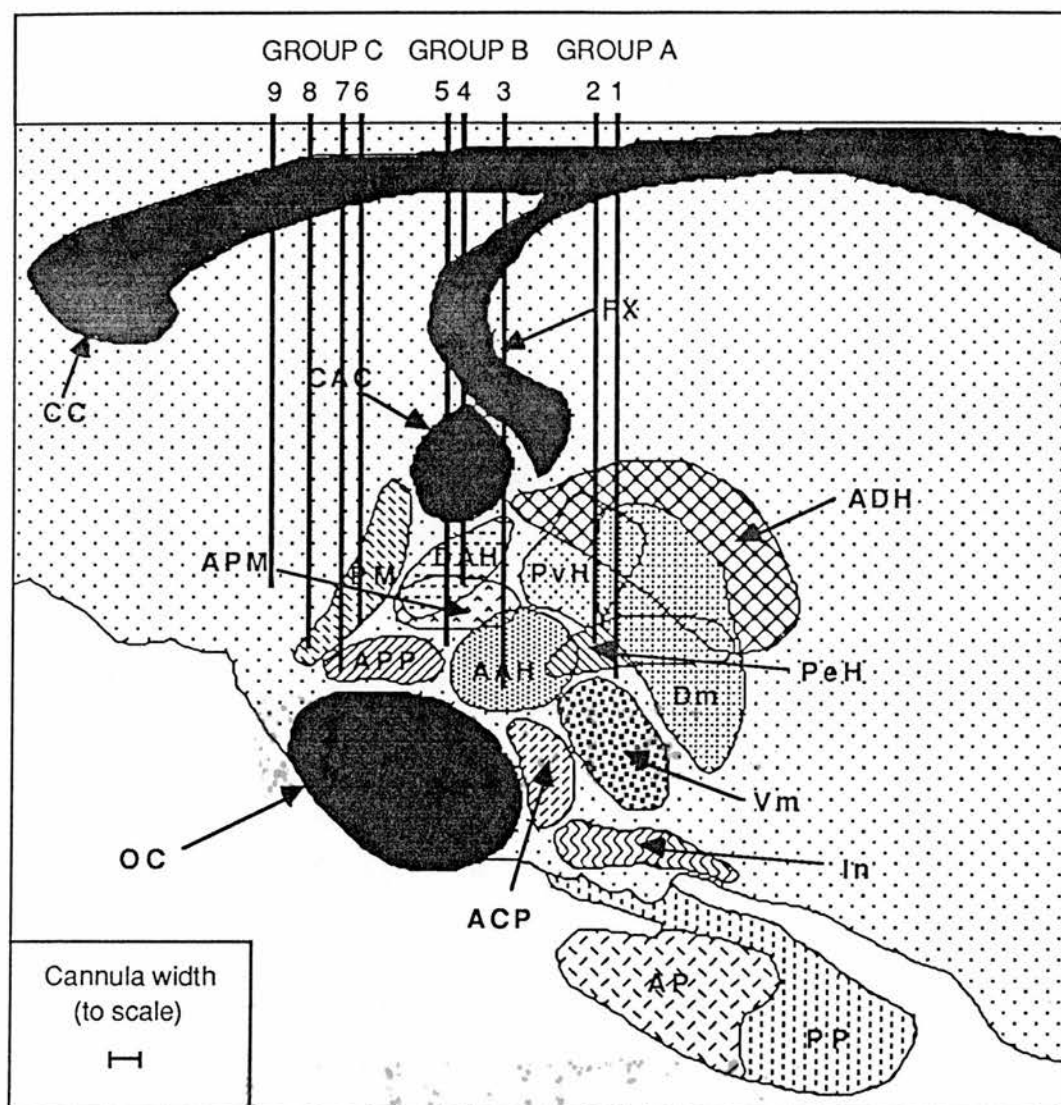


Fig. 5.3 Position of cannulae tips for groups A, B, and C (sagittal section) - compare with Fig. 1.3 for details.

Key:

CC	Corpus callosum	FX	Fornix
CAC	Anterior commissure	ADH	Dorsal hypothalamic area
DAH	Dorsalis anterior nucleus	* PM	Nucleus preopticus medianus
PvH	Paraventricular nucleus	* APM	Medial preoptic area
* APP	Preoptic periventricularis nucleus	AAH	Anterior hypothalamic area
PeH	Periventricular nucleus	OC	Optic chiasm
ACP	Area commissurae postopticae	Vm	Ventromedial nucleus
Dm	Dorsomedial nucleus	In	Arcuate nucleus
AP	Anterior pituitary	PP	Posterior pituitary

* these structures together with the LPOA form the 'POA'

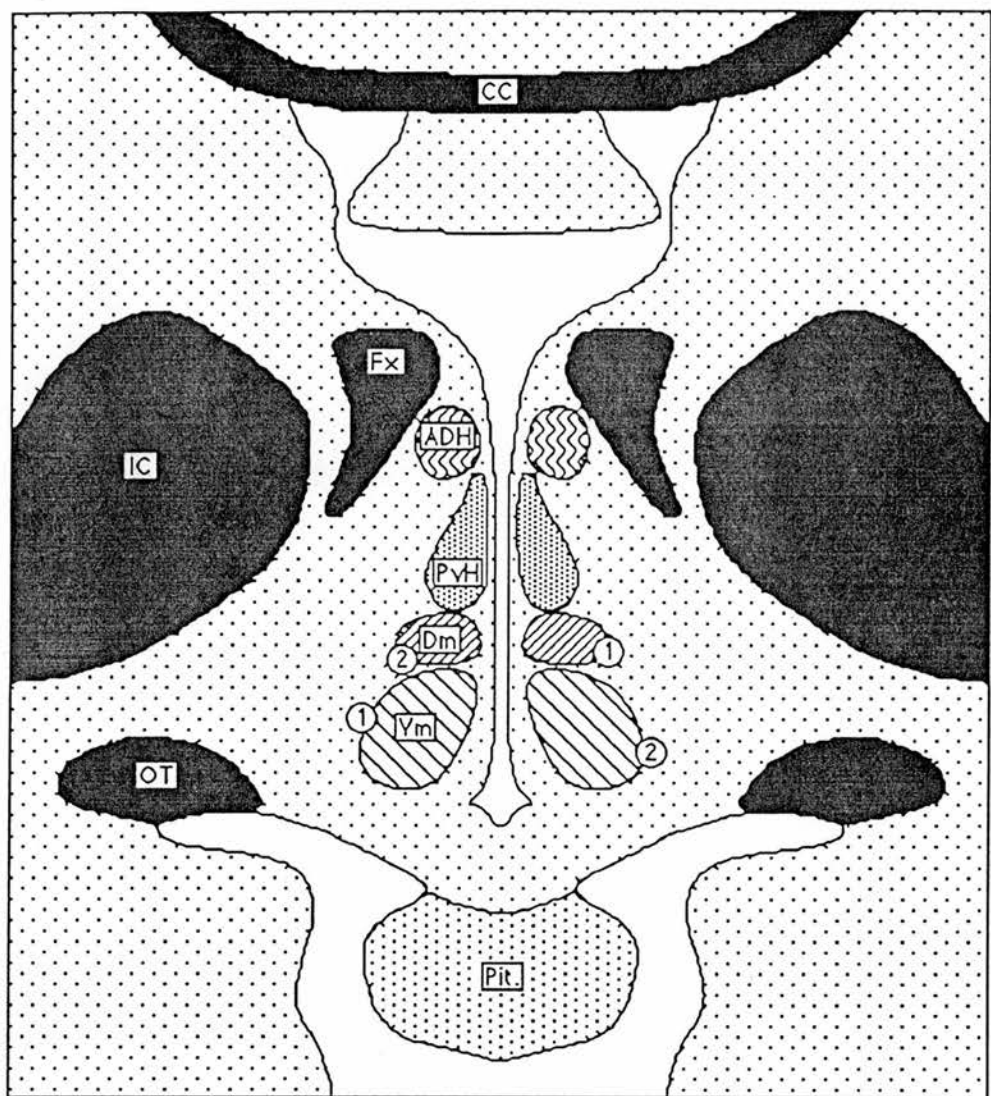


Fig. 5.4a Cannulae placements for Group A (male nos. 1 and 2) - frontal section.

Circles refer to the cannula tip for the male number shown inside. Diameter of circle = width of cannula tip. AP = 9.4.

CC = Corpus callosum
 IC = Internal capsule
 PvH = Paraventricular nucleus
 Vm = Ventromedial nucleus
 Pit. = Pituitary

Fx = Fornix
 ADH = Anterodorsal nucleus
 Dm = Dorsomedial nucleus
 OT = Optic tract

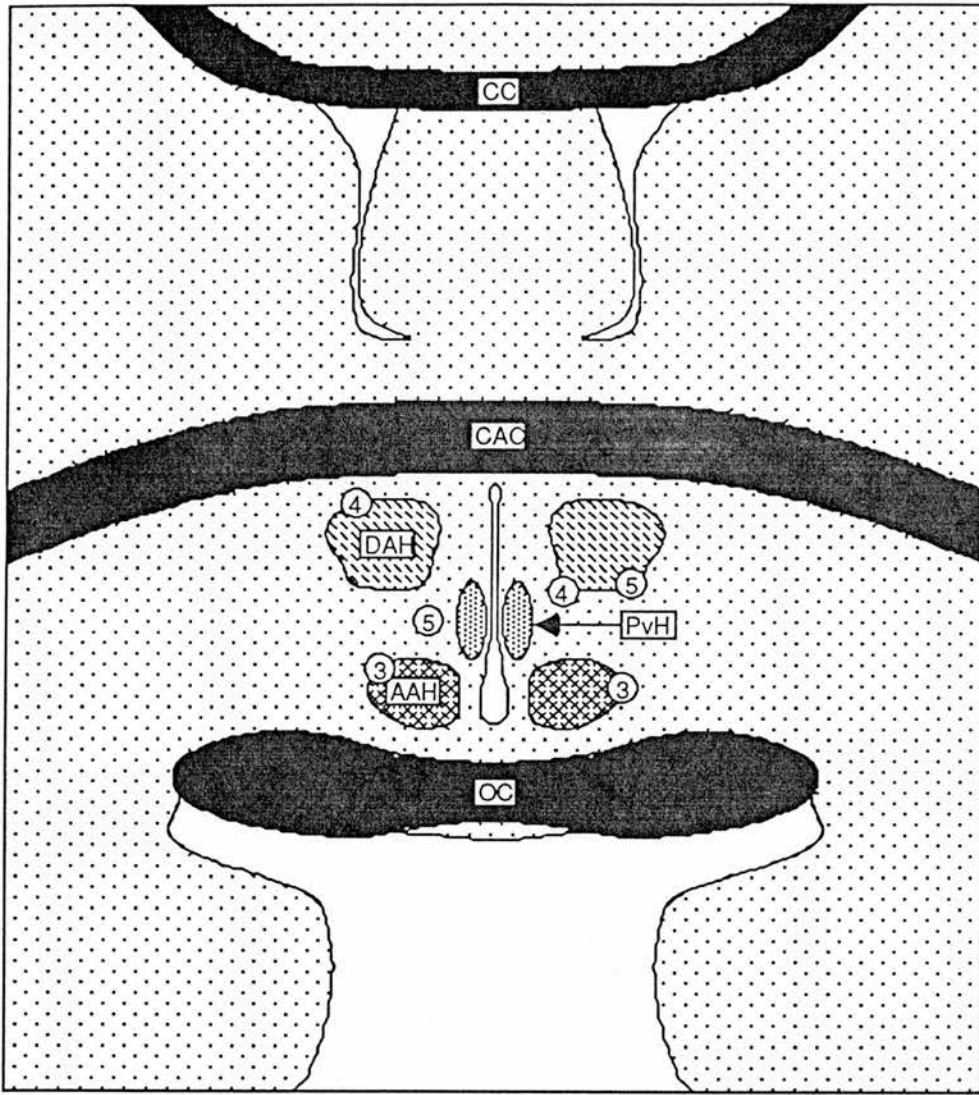


Fig. 5.4b Cannulae placements for Group B (male nos. 3, 4 and 5) - frontal section.

Circles refer to the cannula tip for the male number shown inside. Diameter of circle = width of cannula tip. AP = 9.8.

CC = Corpus callosum

DAH = Dorsalis anterior nucleus

AAH = Anterior hypothalamic area

CAC = Anterior commissure

PvH = Paraventricular nucleus

OC = Optic chiasm

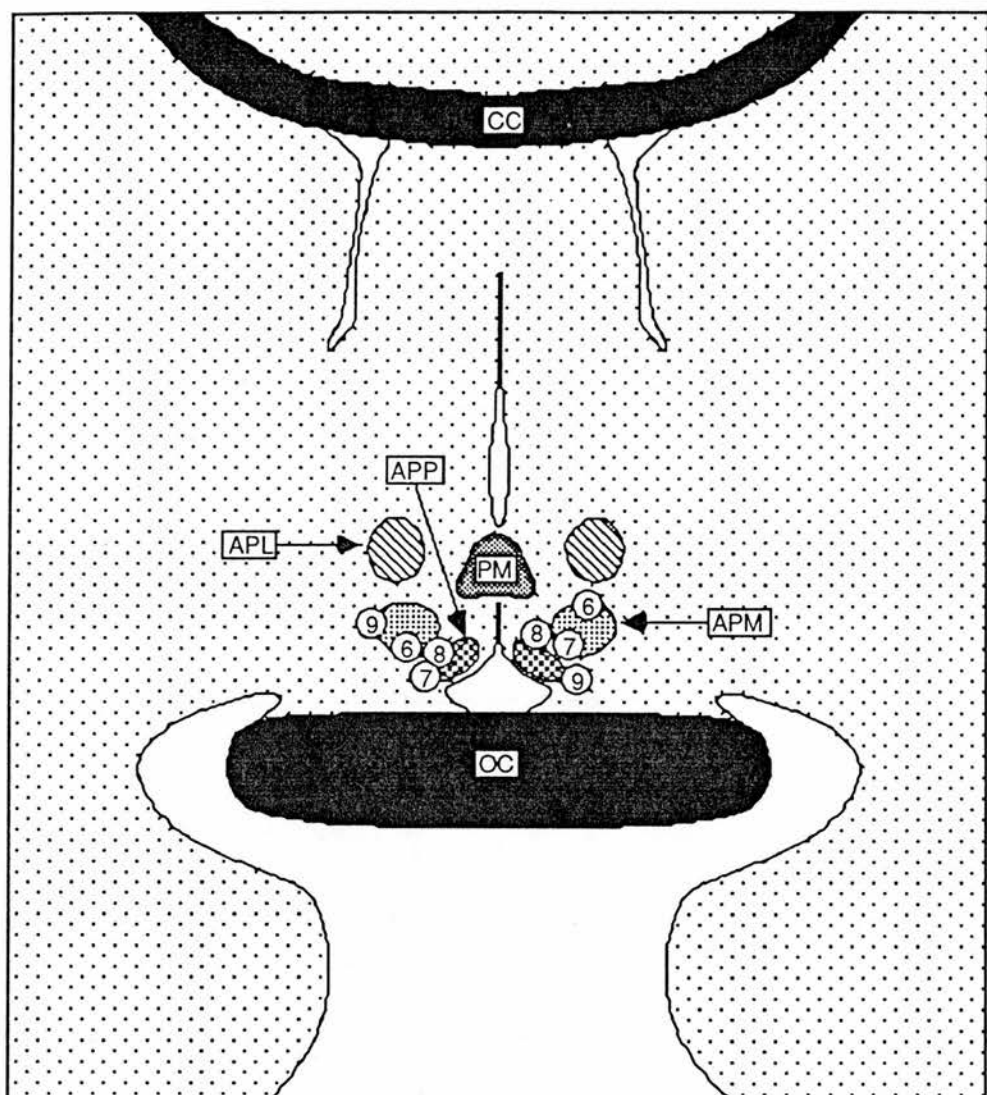


Fig. 5.4c Cannulae placements for Group C (male nos. 6, 7, 8 and 9) - frontal section.

Circles refer to the cannula tip for the male number shown inside. Diameter of circle = width of cannula tip. AP = 10.3.

CC = Corpus callosum	APL = Lateral preoptic nucleus
PM = Nucleus preopticus medianus	APM = Medial preoptic area
APP = Preoptic periventricularis nucleus	OC = Optic chiasm

Statistical analysis within-animals was impossible in this study due to the small number of peptide and control infusions that each male experienced (limited by the possibility of tissue damage following repeated infusions). Where possible, Wilcoxon tests were carried out between males to investigate the effects of infusions of peptide or vehicle into the hypothalamus. Unfortunately, no more precise analysis was possible due to the scatter of cannula placement and the small number of animals in each sub-group, again caused by stereotaxy problems.

5.3.3 Effects of central administration of oxytocin

Fig. 5.5 shows the effects of central administration of oxytocin on the duration of erection during tests for 8 males (male nos. 1, 2, 3, 4, 6, 7, 8 and 9). A Wilcoxon test across the 8 males reveals a significant increase in erection duration for the tests following infusions of 100ng oxytocin compared to the control saline infusions (mean \pm s.e.m. from 633.8 \pm 146.0 to 795.8 \pm 159.1, $n=8$, $T=2$, $P<0.05$). There were no significant effects following the 50ng dose however.

There were no further significant effects on any other aspects of masculine sexual arousal and copulatory activity following infusions of either 50ng or 100ng oxytocin - the individual results for the number of mounts per test are shown in Fig. 5.6.

When other behaviours are considered, Wilcoxon tests across males reveal a significant increase in the number of scent marks per test following infusions of 100ng oxytocin (mean \pm s.e.m. from 9.13 \pm 3.66 to 17.25 \pm 2.96, $n=7$, $T=0$, $P<0.05$) though not following the 50ng dose. The results for the individual males are shown in Fig. 5.7. The only other behaviour that revealed any significant changes on administration of oxytocin was that of male allogrooming. However, in this case, the 50ng infusion resulted in a

significant rise in grooming (mean \pm s.e.m. from 4.94 \pm 1.31 to 6.63 \pm 1.51, $n=8$, $T=4$, $P<0.05$) whilst the 100ng infusion had no effect. It is therefore unlikely that the effects seen here were due to the oxytocin infusions and probably reflect the natural variation in behaviours between successive pair tests. None of the other behaviours measured were altered significantly across the 8 males. No individual statistics were possible due to the small number of tests carried out on each male, but there were no clear trends visible towards behavioural changes within any of the 3 groups for any of the behaviours other than those listed above - see Fig. 5.8.

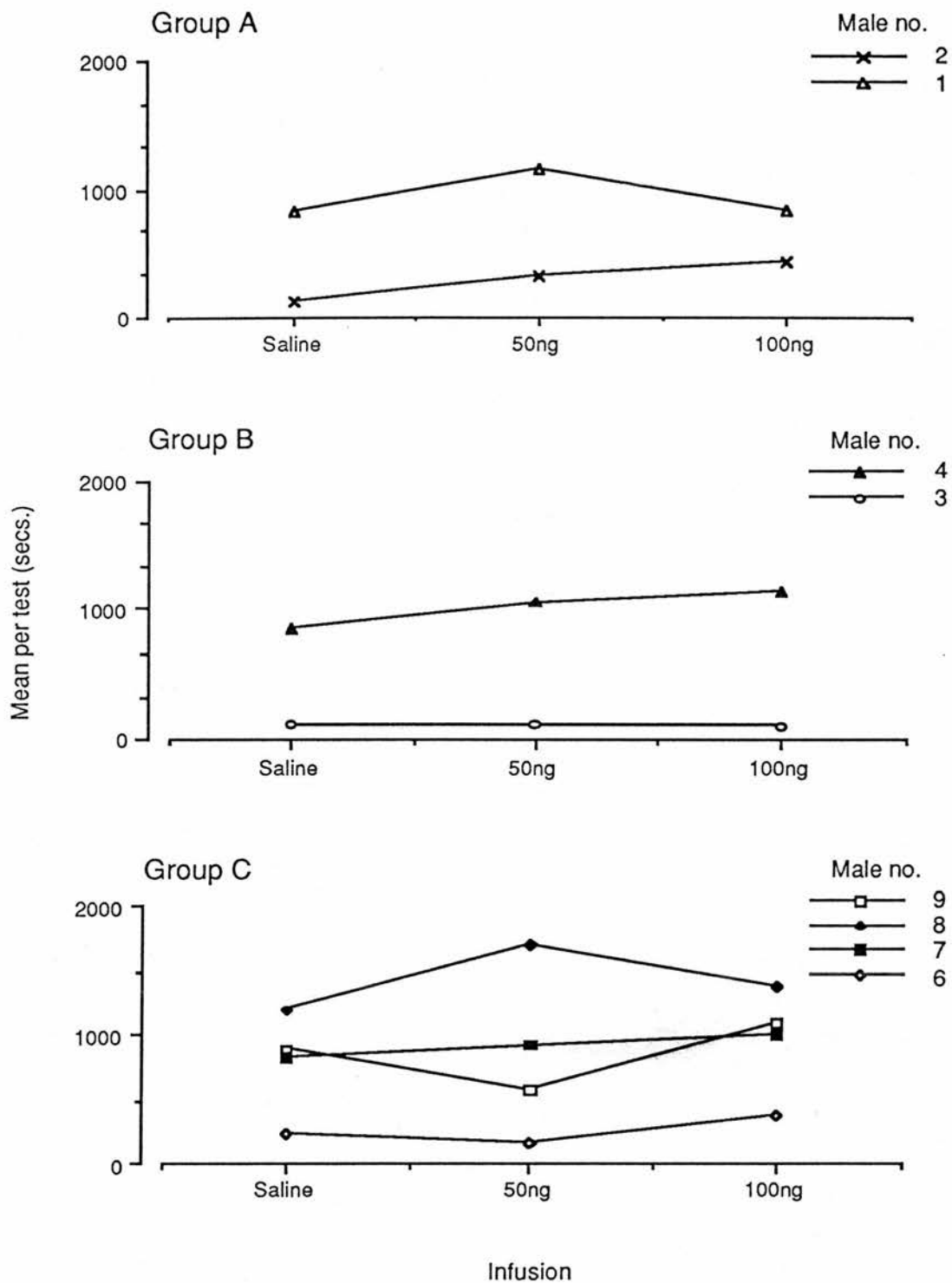


Fig. 5.5 Effects of central administration of saline and oxytocin on erection duration in male marmosets.

Data are plotted for individual males in each group following intracerebral infusions of saline, 50ng and 100ng oxytocin.

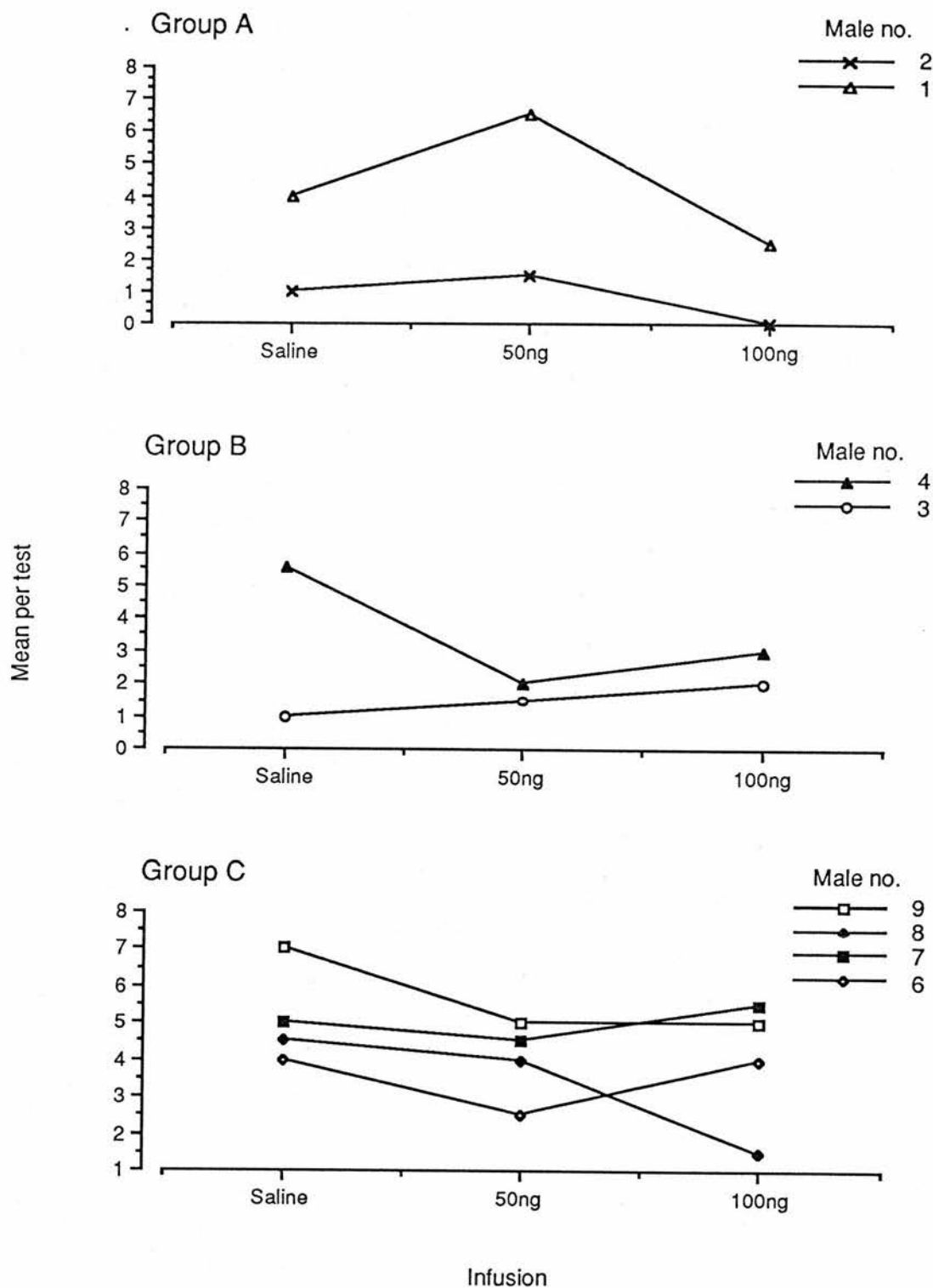


Fig. 5.6 Effects of administration of saline and oxytocin on the number of mounts per test in male marmosets.

Data are plotted for individual males in each group following intracerebral infusions of saline, 50ng and 100ng oxytocin.

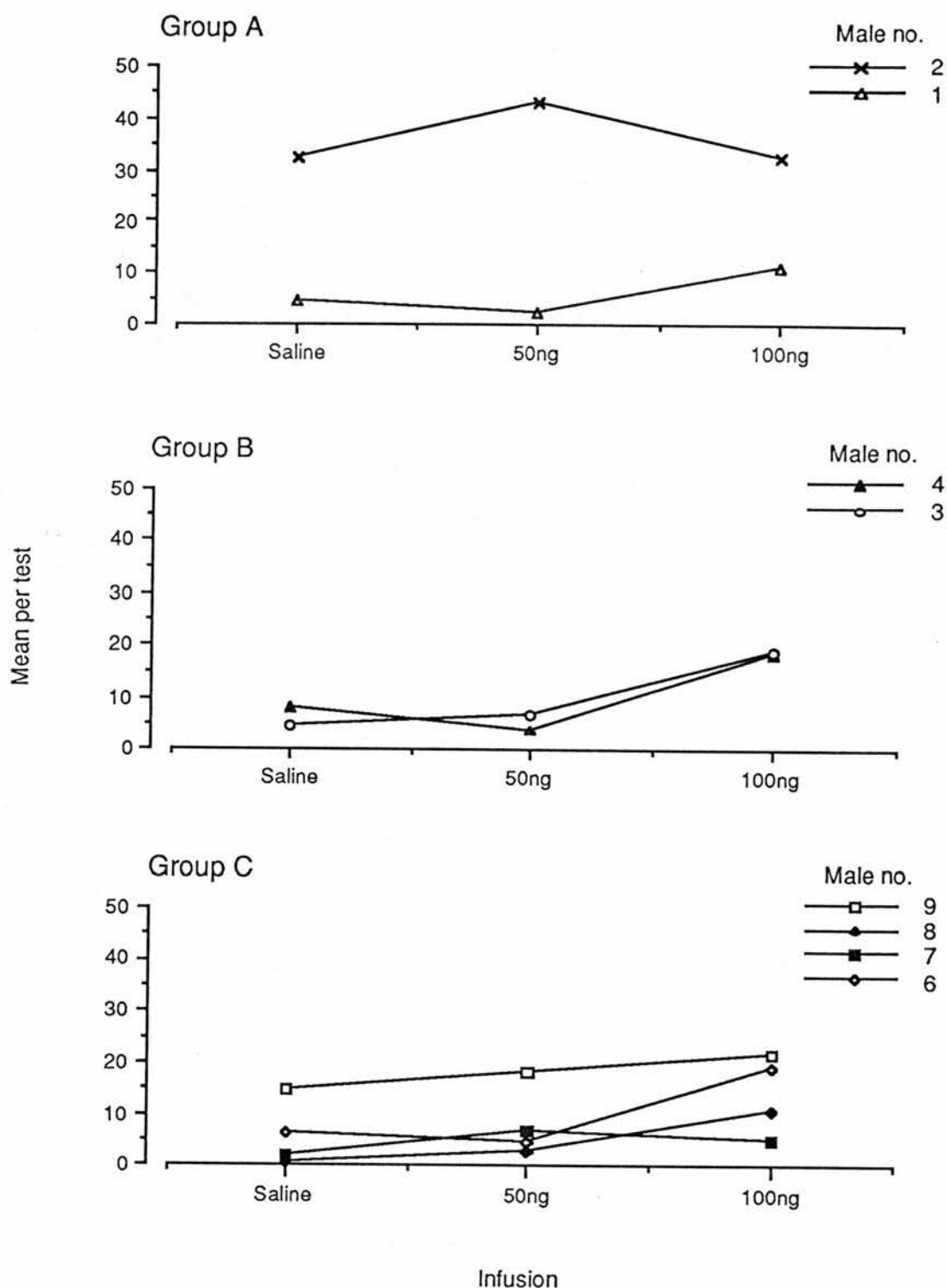


Fig. 5.7 Effects of administration of saline and oxytocin on the number of scent marks per test in male marmosets.

Data are plotted for individual males in each group following intracerebral infusions of saline, 50ng and 100ng oxytocin.

	AG investigation of female			Male proceptive bouts		
Male	Saline	50 ng	100 ng	Saline	50 ng	100 ng
1	0.5 +- 0.35	0.0 +- 0.00	0.5 +- 0.35	2.0 +- 0.71	2.0 +- 0.00	2.0 +- 0.71
2	1.5 +- 1.06	0.5 +- 0.35	1.0 +- 0.71	1.0 +- 0.71	2.5 +- 0.35	0.0 +- 0.00
3	2.0 +- 0.71	3.5 +- 1.77	2.0 +- 0.00	4.0 +- 0.00	1.5 +- 0.71	0.5 +- 0.35
4	1.0 +- 0.00	2.5 +- 1.77	1.0 +- 0.00	0.5 +- 0.35	0.0 +- 0.00	0.0 +- 0.00
6	0.0 +- 0.00	0.0 +- 0.00	0.0 +- 0.00	8.0 +- 0.00	4.5 +- 2.48	9.0 +- 2.12
7	1.0 +- 0.00	1.0 +- 0.00	1.5 +- 0.35	3.0 +- 0.71	1.0 +- 0.71	2.0 +- 0.71
8	8.5 +- 0.35	10.0 +- 0.71	6.5 +- 1.77	0.0 +- 0.00	0.5 +- 0.35	0.0 +- 0.00
9	1.0 +- 0.71	2.0 +- 1.41	0.5 +- 0.35	5.5 +- 1.06	7.0 +- 2.83	1.5 +- 0.35
	No. intromissions			Scent marking		
Male	Saline	50 ng	100 ng	Saline	50 ng	100 ng
1	0.5 +- 0.35	0.5 +- 0.35	1.5 +- 0.35	14.5 +- 0.35	18.0 +- 2.83	22.0 +- 3.54
2	1.0 +- 0.00	1.5 +- 0.35	1.0 +- 0.00	0.5 +- 0.35	2.5 +- 1.06	11.0 +- 3.54
3	1.0 +- 0.00	1.5 +- 0.35	1.5 +- 0.35	2.0 +- 0.71	7.0 +- 4.24	5.0 +- 3.54
4	1.0 +- 0.00	1.0 +- 0.00	1.0 +- 0.00	6.5 +- 0.35	4.5 +- 0.35	19.0 +- 9.90
6	1.5 +- 0.35	1.5 +- 0.35	2.0 +- 0.00	8.0 +- 2.83	4.0 +- 1.41	13.5 +- 4.60
7	1.0 +- 0.71	0.0 +- 0.00	0.5 +- 0.35	4.5 +- 1.77	7.0 +- 2.12	19.0 +- 3.54
8	1.0 +- 0.00	0.5 +- 0.35	1.0 +- 0.00	32.5 +- 4.60	43.0 +- 0.71	32.5 +- 18.0
9	1.0 +- 0.00	2.0 +- 0.00	2.0 +- 0.00	4.5 +- 2.48	2.0 +- 0.71	11.0 +- 4.95
	Male allogrooming			Male grooming invitations		
Male	Saline	50 ng	100 ng	Saline	50 ng	100 ng
1	4.5 +- 0.35	6.0 +- 0.00	4.5 +- 1.06	0.0 +- 0.00	0.0 +- 0.00	0.5 +- 0.35
2	1.0 +- 0.00	0.0 +- 0.00	4.0 +- 0.00	0.0 +- 0.00	0.0 +- 0.00	0.0 +- 0.00
3	8.5 +- 1.77	10.0 +- 1.41	4.0 +- 1.41	1.5 +- 1.06	2.0 +- 1.41	1.0 +- 0.00
4	8.0 +- 0.00	9.0 +- 5.66	2.5 +- 1.06	0.0 +- 0.00	0.0 +- 0.00	0.0 +- 0.00
6	0.0 +- 0.00	2.0 +- 1.41	2.0 +- 0.71	0.0 +- 0.00	0.0 +- 0.00	0.0 +- 0.00
7	5.5 +- 1.77	12.5 +- 0.35	7.5 +- 1.77	0.0 +- 0.00	0.0 +- 0.00	0.0 +- 0.00
8	2.0 +- 0.00	4.5 +- 1.77	4.5 +- 0.35	0.0 +- 0.00	0.0 +- 0.00	0.0 +- 0.00
9	10.0 +- 0.00	9.0 +- 2.83	7.5 +- 1.77	1.0 +- 0.00	0.5 +- 0.35	0.0 +- 0.00

Fig. 5.8 Effects of administration of saline and oxytocin on a number of behaviours shown by male common marmosets.

Data are quoted for individual males following intracerebral infusions of physiological saline, 50ng and 100ng oxytocin.

5.3.4 Effects of central administration of β -endorphin

Statistical analysis of the data either across or within animals was impossible due to the small number of males ($n=5$) and tests (see section 5.2.4) involved. However, no trends across or within groups were seen for any of the behaviours scored, though there is a suggestion of longer mount latencies following infusions of 40pmols β -endorphin. One would expect β -endorphin to exert an inhibitory influence on sexual behaviour (Hughes et al., 1987), however, Fig. 5.9 indicates that this is not the case for either the number of mounts per test or for mount latency.

	Number of mounts		
Male	Artificial CSF	40pmols.	80pmols.
2	3.25 \pm 0.89	3.50 \pm 0.35	5.00 \pm 2.12
3	2.25 \pm 0.54	2.50 \pm 0.35	1.50 \pm 0.35
4	5.00 \pm 0.79	4.00 \pm 1.41	5.00 \pm 0.00
6	2.50 \pm 0.56	3.00 \pm 1.41	3.00 \pm 0.00
7	1.25 \pm 0.22	2.00 \pm 0.71	2.50 \pm 0.35
	Mount Latency		
Male	Artificial CSF	40pmols.	80pmols.
2	5.5 \pm 0.25	21.0 \pm 11.32	4.5 \pm 0.4
3	3.0 \pm 0.35	5.5 \pm 1.06	3.0 \pm 0.0
4	2.0 \pm 0.00	3.0 \pm 0.00	2.0 \pm 0.0
6	54.0 \pm 128.5	513.0 \pm 20.5	206.0 \pm 140
7	9.5 \pm 0.79	97.5 \pm 61.88	55.5 \pm 32.7

Fig. 5.9 Effects of administration of artificial CSF and β -endorphin on the number of mounts per test and mount latency in male marmosets.

Data are quoted for individual males (nos. 2, 3, 4, 6 and 7) following intracerebral infusions of artificial CSF, 40pmols and 80pmols β -endorphin.

Grooming behaviour has also been seen to be affected by opiates in other studies (see Discussion - Chapter 4) and one would expect to see decreases in both male allogrooming and grooming invitations in the present study. However, both these behaviours occurred at low levels throughout the test series thus masking any possible effects of β -endorphin - see Fig. 5.10.

	Male allogrooming		
Male	Artificial CSF	40pmols.	80pmols.
2	1.75 +- 0.22	0.00 +- 0.00	1.00 +- 0.71
3	0.75 +- 0.41	1.50 +- 0.35	0.50 +- 0.35
4	0.75 +- 0.41	0.00 +- 0.00	0.00 +- 0.00
6	0.00 +- 0.00	0.00 +- 0.00	0.00 +- 0.00
7	4.00 +- 0.94	3.50 +- 1.77	3.50 +- 0.35
	Male grooming invitations		
Male	Artificial CSF	40pmols.	80pmols.
2	0.00 +- 0.00	0.00 +- 0.00	0.00 +- 0.00
3	0.25 +- 0.22	0.00 +- 0.00	0.00 +- 0.00
4	0.00 +- 0.00	0.00 +- 0.00	0.00 +- 0.00
6	0.00 +- 0.00	0.00 +- 0.00	0.00 +- 0.00
7	0.00 +- 0.00	0.00 +- 0.00	0.00 +- 0.00

Fig. 5.10 Effects of administration of artificial CSF and β -endorphin on allogrooming and grooming invitations in male marmosets.

Data are quoted for individual males (nos. 2, 3, 4, 6 and 7) following intracerebral infusions of artificial CSF, 40pmols and 80pmols β -endorphin.

5.3.5 Effects of repeated infusions

4 males (nos. 1, 5, 8 and 9) stopped showing sexual behaviour either before or during the β -endorphin testing series. Male no. 1 failed to intromit or

ejaculate on a test following an infusion of β -endorphin and on any of the subsequent tests. This male had been given 8 infusions at this time. Male no. 5 failed to mount on a test following an infusion of artificial CSF and on any subsequent tests. This male had been given 11 infusions at this time. Male no. 8 also stopped mounting following an infusion of artificial CSF and this was this animal's 7th infusion. Finally, male no. 9 continued to mount at high rates but, following an infusion of artificial CSF (the 10th infusion for this animal) intromissions occurred only infrequently. This was assumedly due to repeated infusions as all 4 males remained in good health throughout the course of the experiment. From the histology, there was no evidence of any difference in the tissue surrounding the cannulae tips of these males compared with the 5 remaining males - see Fig. 5.11.



Fig. 5.11 Photograph of a representative frontal section of male no. 1 showing the cannulae tracts.

The cessation of sexual activity occurred abruptly in all cases and therefore the males were given a number of pair tests in an attempt to elucidate the precise deficit exhibited. No recovery of sexual behaviour was seen during

these tests. No statistical analysis of the data was possible due to the small number of tests in some cases, though the data shown in Fig. 5.12 give some indication of the effects of tissue damage within the areas shown in Fig. 5.1.

	No. mounts		Mount latency (secs.)	
Male	Pre	Post	Pre	Post
1	3.3 +- 0.66	0.4 +- 0.22	4.7 +- 0.63	793 + 26 *
5	9.5 +- 1.06	0.0 +- 0.00	18.0 +- 7.07	- - -
8	1.3 +- 0.22	0.0 +- 0.00	3.0 +- 0.35	- - -
9	5.7 +- 0.45	6.3 +- 0.84	2.8 +- 0.76	2.8 +- 0.28
	Erection duration (secs.)		Anticipatory erection (%)	
1	734.2 +- 104.83	96.2 +- 34.66	70.0 +- 14.00	40.0 +- 0.22
5	894.0 +- 4.24	0.0 +- 0.00	50.0 +- 35.00	0.0 +- 0.00
8	222.8 +- 59.49	119.3 +- 110.43	0.0 +- 0.00	0.0 +- 0.00
9	854.8 +- 111.30	611.7 +- 80.87	100.0 +- 0.00	100.0 +- 0.00
	Male precopulatory bouts		AG investigation of female	
1	3.0 +- 1.06	0.4 +- 0.22	0.8 +- 0.40	0.0 +- 0.00
5	0.0 +- 0.00	0.0 +- 0.00	1.0 +- 0.71	0.0 +- 0.00
8	0.3 +- 0.22	0.3 +- 0.17	9.3 +- 0.54	5.7 +- 1.16
9	2.0 +- 0.33	0.5 +- 0.20	0.3 +- 0.19	2.7 +- 0.38
	Male allogrooming		No. intromissions	
1	5.9 +- 1.38	0.0 +- 0.00	1.8 +- 0.13	0.0 +- 0.00
5	4.0 +- 0.00	0.0 +- 0.00	0.5 +- 0.35	0.0 +- 0.00
8	3.3 +- 1.08	2.7 +- 0.72	0.5 +- 0.25	0.0 +- 0.00
9	5.0 +- 0.47	3.0 +- 0.67	0.8 +- 0.28	0.3 +- 0.19

Fig. 5.12 Effects of repeated intracerebral infusions upon a range of behaviours shown by male common marmosets.

Data are mean +- s.e.m. for individual males (nos. 1, 5, 8 and 9) before (pre) and after (post) the test on which sexual activity ceased or dramatically declined. * = only 2 post-operative mounts, therefore individual times are quoted.

Male no. 1, whose cannulae were positioned in the medial hypothalamus, suddenly began to mount at very low frequencies and no further intromissions were seen. This male also showed little social interaction with his female partner following the loss of sexual activity as evidenced by the absence of grooming behaviour. This lack of interaction was also apparent in the case of male no. 5, whose cannulae were positioned within the AH, in which very little social behaviour occurred following the loss of sexual activity. Male no. 8 also showed a loss of mounting behaviour though continued to allogroom and to investigate the anogenital region of his female partner and to exhibit precopulatory tongue flicking. The cannulae in this male were situated within the POA. Finally, male no. 9 continued to mount at a high rate, but showed a decline in the number of mounts which were followed by intromissions, though all other aspects of his behaviour continued at normal frequencies. This male's cannulae were placed within the POA.

5.4 Discussion

This study is open to the same criticisms that can be levelled at the data presented in the preceding chapter. Due to the inaccuracy of the stereotaxic procedure, the cannulae were implanted in the males at differing locations. Again, this precluded the use of an analysis of variance with respect to cannula placement.

The original design of this experiment was that the cannulae in all the 9 males should be situated beneath the anterior commissure, at the junction of the AH and the POA - in the area in which lesions in the previous experiment appeared to have maximal effects on sexual behaviour. If this had been the

case, the role of oxytocin and β -endorphin within this specific area could have been elucidated by means of statistical analysis.

However, this bilateral cannulation technique has not been carried out in this species before and this study at least presents evidence that this procedure is practicable in the common marmoset.

The results from the first part of the study were disappointing as the plasma levels of oxytocin in both males and females appear to be below the detection limit of the assay. It is therefore impossible to state whether ejaculation or vaginocervical stimulation effects plasma oxytocin levels in the common marmoset. When considering data from other studies, the changes measured in plasma levels following these manipulations are considerably smaller than those that would have been detectable using the protocol described here (Ogawa et al., 1980; Seckl & Lightman, 1987; Carmichael et al., 1987). If changes in plasma oxytocin are to be measured in the common marmoset, it will be necessary to develop a more sensitive assay.

The results from the intracerebral cannulation studies are interesting for a number of reasons. Oxytocin has been found to stimulate spontaneous erections in male rats following infusions of 3-27ng/0.3 μ l vehicle into the PvH (Melis et al., 1986), and to increase mount and intromission latencies and the post-ejaculatory interval following infusions of 250ng/2 μ l vehicle into the third ventricle of rats (Stoneham et al., 1985). It was therefore expected that some behavioural effects would be seen following infusions of 50-100ng/0.5 μ l vehicle into the PvH of common marmosets. From studying the cannulae placements (Fig. 5.4a-c), it is clear that the males in Groups A and B would have received infusions of oxytocin into the anteromedial hypothalamus adjacent to the PvH. One would therefore expect to see behavioural effects following infusions in at least these males if the PvH does indeed influence penile erection or copulatory behaviour. It is

interesting that the most noticeable change in behaviour following oxytocin infusions was a significant increase in erection duration following infusions of 100ng oxytocin. This would therefore appear to be in agreement with the data from rats (Melis et al., 1986). Interestingly, no effects on other aspects of sexual behaviour, such as mount or intromission latencies, were seen. However, in the previous study (Stoneham et al., 1985), oxytocin was infused into the third ventricle from which it can diffuse throughout the entire brain; thus the effects on copulatory behaviour may have been mediated at sites distant from the MPOA-AH.

The results following infusions of β -endorphin are more difficult to explain. It is unfortunate that only 5 males were able to continue through this phase of the study rendering statistical analysis impossible. However, if intracerebral infusions of β -endorphin do indeed influence aspects of behaviour at these doses, it should be possible to determine trends in the data. As previously mentioned, infusions of β -endorphin into the MPOA-AH of male rats (5-40pmols/0.5 μ l vehicle) cause significant declines in sexual behaviour (Hughes et al., 1987). The cannulae of the males in Groups B and C were adjacent to the MPOA-AH and, therefore, β -endorphin infused into these males could be expected to influence sexual behaviour. However, it is clear from the data quoted that the doses and volumes used in this study did not affect any aspect of the males' sexual behaviour.

There are a number of reasons which might explain why this is the case. It is unlikely that the dose was ineffective as the levels were based on 1x and 2x the most effective rat dose. However, it is possible that the volume of vehicle used was too small to ensure adequate diffusion. Unfortunately, in designing the experiment, the volume of vehicle used was balanced against the possible adverse effects of local tissue damage following infusions of large volumes. A further possibility is that the distribution of β -endorphin

neurons varies between rats and marmosets. It is known that the MPOA-AH of primates contains high concentrations of β -endorphin (Matsukura et al., 1978; Fuchs et al., 1986) but it is possible that the projections of the neurons are different.

As previously mentioned, administration of opiate antagonists stimulates affiliative behaviour in talapoin (Fabre-Nys et al., 1982). The authors conclude that the state of endogenous opiate withdrawal induced by these antagonists caused the monkeys to seek support and comfort in grooming behaviour with other individuals. Following this argument through, administration of exogenous opiates may lead to a decreased need for social interactions in the form of allogrooming and grooming invitations in the present study. Unfortunately, these behaviours occurred at relatively low rates during the tests following control infusions, thus masking any possible effects of β -endorphin. Furthermore, in the previous study, the opiate antagonists were administered peripherally and may not have been influencing affiliative behaviour via hypothalamic opiate mechanisms.

The males that ceased to show sexual activity are of interest too. It is assumed that local tissue damage caused by repeated intracerebral infusions resulted in these deficits. Although these males did not receive a larger number of infusions than those that continued to copulate regularly, it is possible that the fluid was infused more forcefully in some cases - though care was taken to ensure that each infusion lasted exactly 1 minute. The behaviour of male nos. 1 and 5 following the deficit closely resembled that of a male given a Kanivacid ~~lesion~~ *lesion* in a pilot study i.e. all three males showed little social interaction of any kind with their female partners. The two males with more rostral cannulae placements (male nos. 8 and 9) appeared to suffer a less generalised social deficit, as both males continued to interact with their females and male no. 9 continued to mount

frequently though with a reduction in the number of mounts culminating in intromission and ejaculation. No firm conclusions can be drawn from these data however, as the precise cause of the deficits is not known. The histology of the males that suffered deficits did not differ from that of the males that continued to exhibit sexual behaviour and therefore these effects may have been caused by totally unrelated factors.

The general conclusion to be drawn from this series of experiments is that an intracerebral cannulation technique is viable in the common marmoset though more work is now required to elucidate the specific effects of the neuropeptides in question on masculine sexual and social behaviours. This new technique will open up many avenues of research into the neural control of behaviour in this primate species and these invasive studies are more feasible using small monkeys such as the marmoset than the large-bodied primates that have been traditionally used in such studies.

CHAPTER 6

EXPERIMENT 3: POA-AH LESIONS AND MASCULINE SEXUAL AND PATERNAL BEHAVIOUR IN A PERMANENT GROUP SITUATION

6.1 Introduction

It is clear from Experiment 1 that the POA-AH is an integral part of the mechanism controlling masculine sexual behaviour in the common marmoset. There is also evidence to implicate this area in the control of maternal behaviour, as reviewed in Chapter 2, and it is possible that the same neural circuits control paternal care. There is also a peak in sexual activity coincident with the female's postpartum ovulation (Dixon & Lunn, 1987) so it was decided to study the effects of POA-AH lesions on paternal behaviour and sexual activity during this postpartum period. By studying behaviour in established breeding pairs, results from a more natural situation could be obtained than can be from a pair test experiment and a thermal lesion technique was employed to provide similar lesions to those in Experiment 1, so that comparisons could be drawn between the two sets of data.

6.2 Materials and methods

6.2.1 Animals

The 7 males and 7 females used in this study were selected using the criteria described in Chapter 3.

In all but one case, twin offspring were produced, though one set of infants died on the 5th day postpartum and this group was removed from the study. In the last case, triplets were produced though one was born dead and was removed from the cage immediately.

6.2.2 Surgery

Males received thermal hypothalamic lesions (n=3) or sham lesions (n=3) as described in Chapter 3. The atlas coordinates used were as follows: AP +10.3mm, V +8.0mm + +9.0mm, L + \pm 0.5mm for the lesioned males and AP +10.3mm, V +10.5mm, L + \pm 0.5mm for the sham lesioned controls.

In all cases, recovery was rapid and the males were returned to the video cage within 3-4 hours of removal.

6.2.3 Video recording

The animals used in this study were monitored using a time-lapse video recording system as outlined in Chapter 3. Behaviour was recorded at 4x normal speed and the tapes were run for the full 12 hours of the light period (0600h - 1800h) each day.

6.2.4 Testing regime

The pairs were placed in the video cage 2-3 weeks prepartum to allow time for habituation to the new environment before parturition. Tapes were recorded each day from this time onwards to ensure that data for the first day postpartum would be collected. In all cases, parturition occurred during the dark period.

Each pair was recorded for 30 days postpartum and the first 5 hours of each day (0600h - 1100h) were scored for all aspects of sexual, social and parental behaviour. In addition, locomotor activity, eating and drinking bouts and scent marking were scored for the first hour of each day for the male.

Surgery was carried out on the afternoon of the 11th day postpartum and the males were returned to the cage on recovery from the anaesthetic. This regime allowed each male to act as his own control, providing 11 days of pre-operative data and 19 days post-operatively. Day 11 was chosen as a

compromise between obtaining the maximum amount of pre-operative data and ensuring that the males were lesioned before or during the post-partum rise in sexual activity.

Male no. 3 was also studied through the subsequent postpartum period with the same female partner for 17 days, though the video system was not used in this instance. For this period, only carrying behaviour was scored by 'spot checks' every half an hour between 0900h and 1700h.

6.2.5 Behaviours scored

The behaviours listed here are defined in detail in Chapter 3.

During the first 5 hours of each day, the position of the twin offspring was recorded i.e. on the female, on the male, or during 'time off' when the infants were not on either parent. For this reason, a total of 10 hours carrying time was recorded (5 hours for each of the twin infants) each day. In addition, transfers, dislodge behaviour, re-establishment of contact, allogrooming and anogenital grooming of both infants were scored. Allogrooming between the adults was also scored, as were mounts, intromissions, intromission latencies (from the first mount), ejaculations and ejaculation latencies for the male and mount initiations, refusals and terminations by the female.

6.2.6 Histology

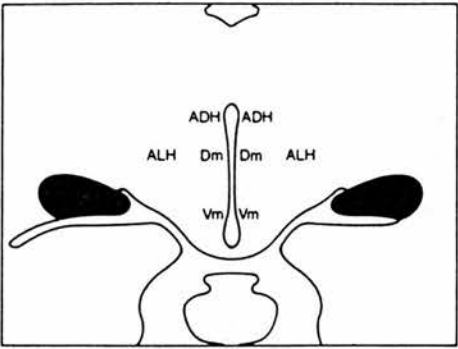
At the conclusion of the study, the brains of the thermally lesioned males were prepared and treated in the manner described for staining with luxol fast blue and cresyl fast violet in Chapter 3. Lesion volumes were then calculated using the image analyser.

6.3 Results

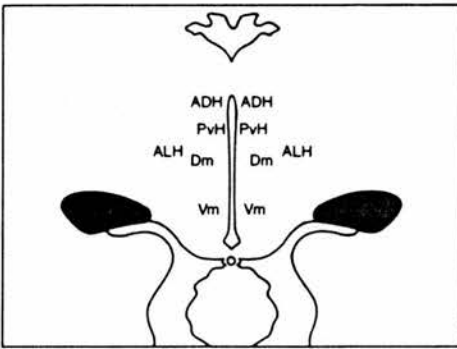
6.3.1 Lesion placement

Fig. 6.1 shows a series of frontal sections of the lesions in each male. Here, and subsequently, the males are numbered 1-3 in order of lesion placement relative to interaural zero i.e. male no. 1 has the most caudal and male no. 3 the most rostral lesion placement. All 3 lesions were bilateral and centred within the POA-AH, and Fig. 6.2 shows the nuclei damaged, the approximate AP, relative to interaural zero at the centre of the lesion, and the lesion volume.

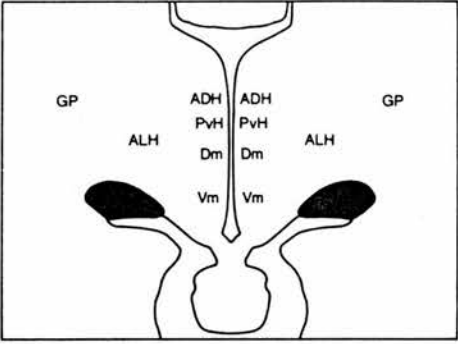
Male no. 1 had a lesion centred on the PM in the POA, though major damage extended caudally into the AH at the level of the dorsalis anterior (DAH) and paraventricular (PvH) nuclei. Male no. 2 received similar damage, though more of the DAH was spared in this instance. In the case of male no. 3, the lesion was again centred in the POA with very little damage caudal to this structure. Lesion volumes varied from 1.45 - 2.79 mm³.



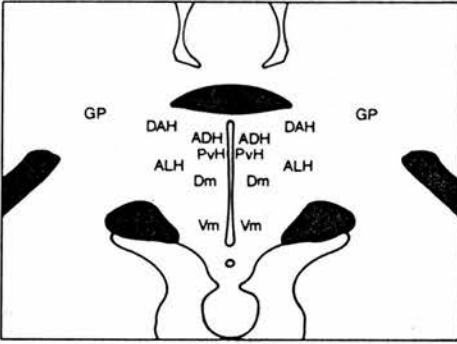
AP 7.5



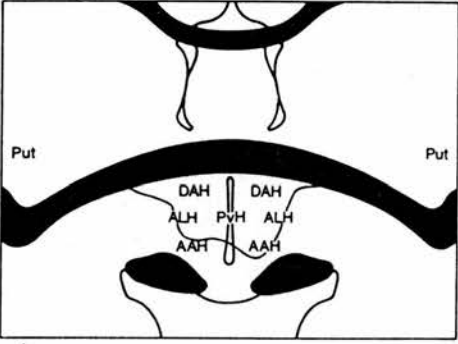
AP 8



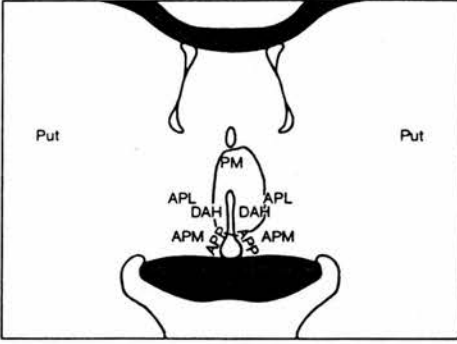
AP 8.5



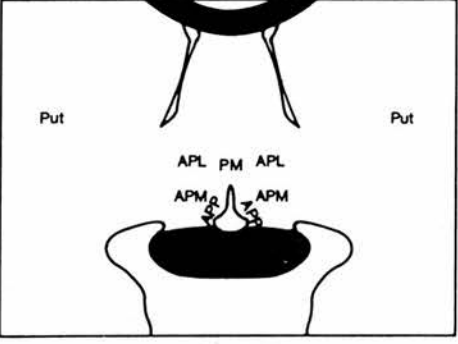
AP 9



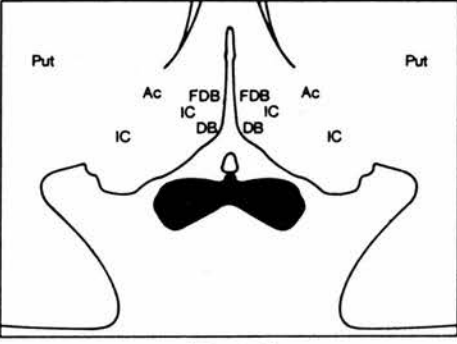
AP 9.5



AP 10

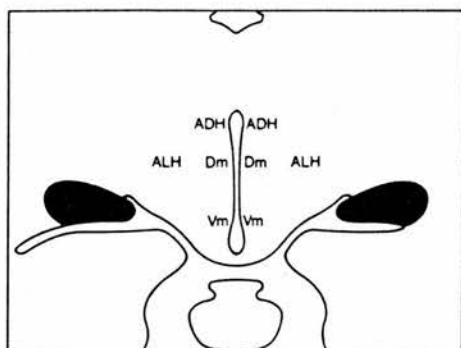


AP 10.5

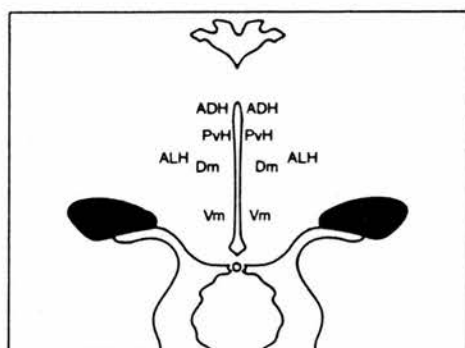


AP 11.5

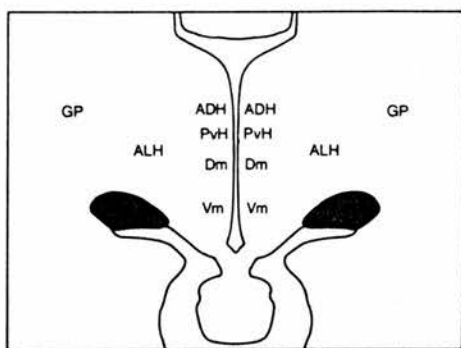
Male No. 1



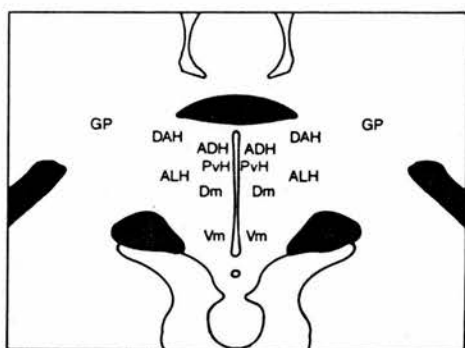
AP 7.5



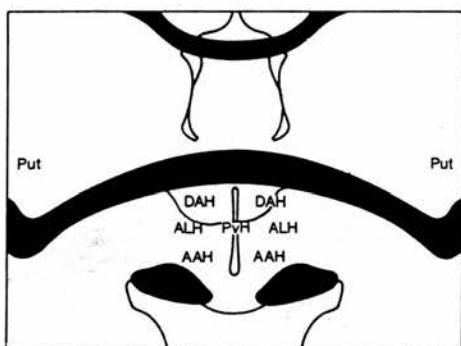
AP 8



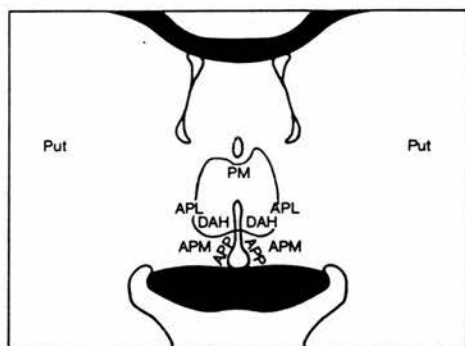
AP 8.5



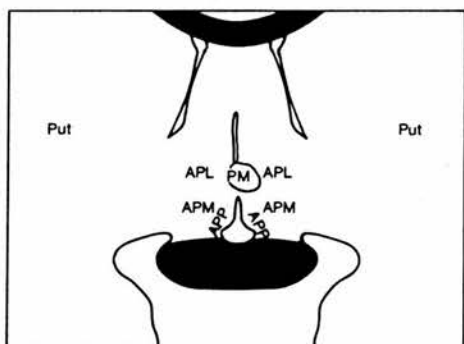
AP 9



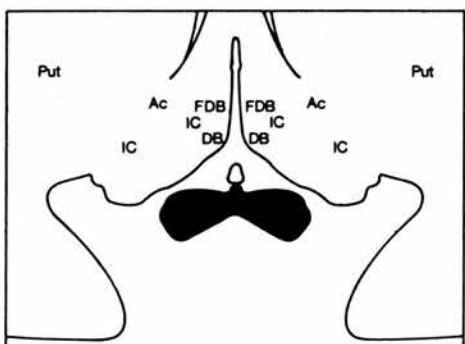
AP 9.5



AP 10

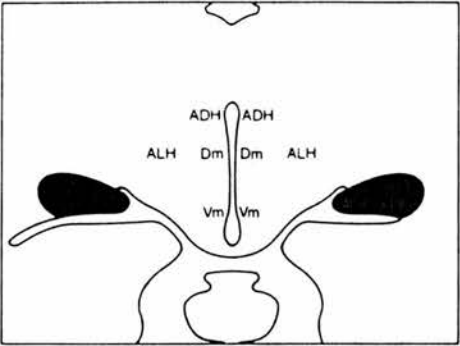


AP 10.5

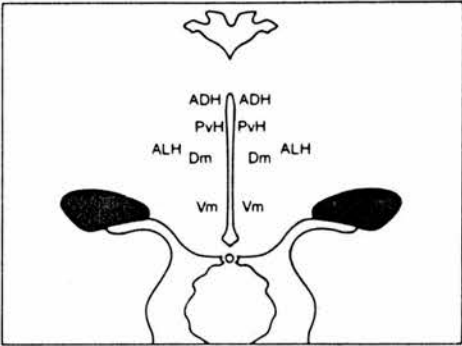


AP 11.5

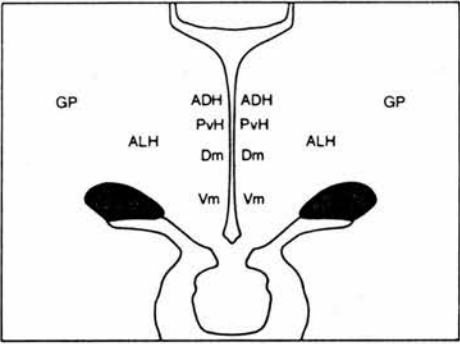
Male No. 2



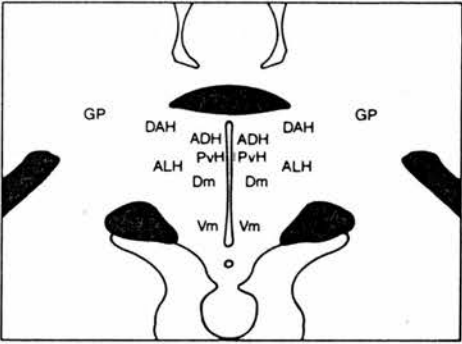
AP 7.5



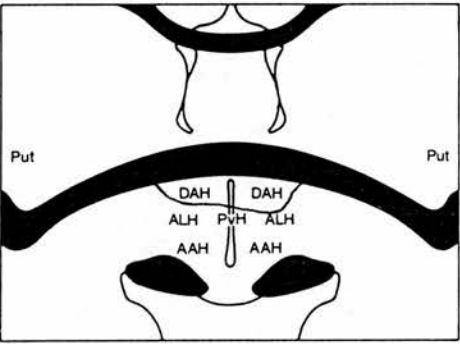
AP 8



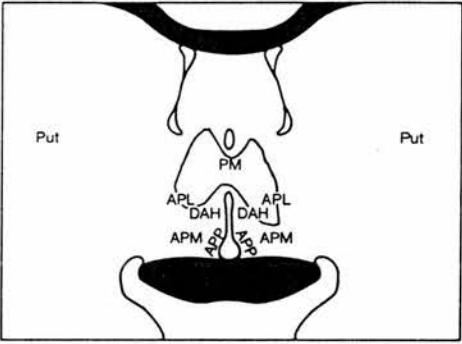
AP 8.5



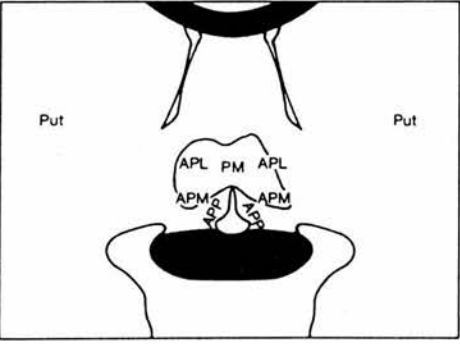
AP 9



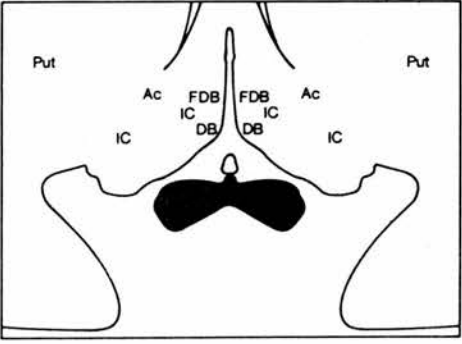
AP 9.5



AP 10



AP 10.5



AP 11.5

Male No. 3

Fig. 6.1 The position and extent of the lesions of male nos. 1-3.

The lesions from each of the males are shown on a series of sections (AP 7.5mm - 11.5mm) taken from the marmoset atlas (Stephan et al., 1980). All sections are shown for each male to facilitate comparison of lesion placement between the 3 individuals and with the lesions described in Chapter 4 (pages 117-126).

Key:-

Vm = ventromedial nucleus
Dm = dorsomedial nucleus
ALH = lateral hypothalamic area
ADH = dorsal hypothalamic area
PvH = paraventricular nucleus
GP = globus pallidus
DAH = dorsalis anterior nucleus
AAH = anterior hypothalamic area
Put = putamen
APP = preoptic periventricularis nucleus
APM = medial preoptic area
APL = lateral preoptic nucleus
PM = nucleus preopticus medianus
DB = diagonalis Brocae
IC = insulae Callejae
FDB = fasciculus diagonalis Brocae
Ac = nucleus accumbens

	OTHER			POA				AH				OTHER			AP AT CENTRE	VOLUME (mm ³)
	DB	IC	FDB	PM	APL	APM	APP	DAH	ALH	AAH	PvH	ADH	Vm	Dm		
1				**	*			***	*		**	*			9.7	2.79
2				**	*			**			**				9.9	2.22
3				***	**	*		*							10.1	1.45

Fig. 6.2 Positions and volumes of hypothalamic lesions in male marmosets (nos. 1-3).

The degree of damage to the individual nuclei is shown, as well as the position of the lesion centre relative to interaural zero (AP), and the total volume of tissue damaged (mm³).

* = slight damage only ** = medium damage *** = substantial damage

DB = diagonalis Brocae

IC = insulae Callejae

FDB = fasciculus diagonalis Brocae

PM = nucleus preopticus
medianus

APL = lateral preoptic nucleus

APM = medial preoptic area

APP = preoptic periventricularis nucleus

DAH = dorsalis anterior nucleus

AAH = anterior hypothalamic area

ALH = lateral hypothalamic area

PvH = paraventricular nucleus

ADH = dorsal hypothalamic area

Vm = ventromedial nucleus

Dm = dorsomedial nucleus

6.3.2 Effects on carrying time

The effects of hypothalamic lesions on carrying time in these males are shown in Fig. 6.3. Mann-Whitney U-tests within-animals revealed significant and dramatic decreases in this behaviour for male no. 1 (mean \pm s.e.m. 33.21 \pm 5.94% to 1.77 \pm 0.90% of total time, $n_1=11$, $n_2=19$, $U=6$, $P<0.001$), male no. 2 (67.69 \pm 3.84% to 25.21 \pm 6.92% of total time, $n_1=11$, $n_2=19$, $U=25$, $P<0.001$) and male no. 3 (82.1 \pm 1.55% to 0.37 \pm 0.07% of total time, $n_1=11$, $n_2=19$, $U=0$, $P<0.001$). No similar decline in the carrying behaviour of the control males (nos. C1-C3) was apparent.

Fig. 6.3 also shows that the females compensate for the lack of male carrying behaviour, thus resulting in significant increases in this behaviour post-operatively. This compensation accounts for the fact that the length of time that the infants spent in 'time off' was similar between the lesioned males' groups and the control males' groups - see Fig. 6.3.

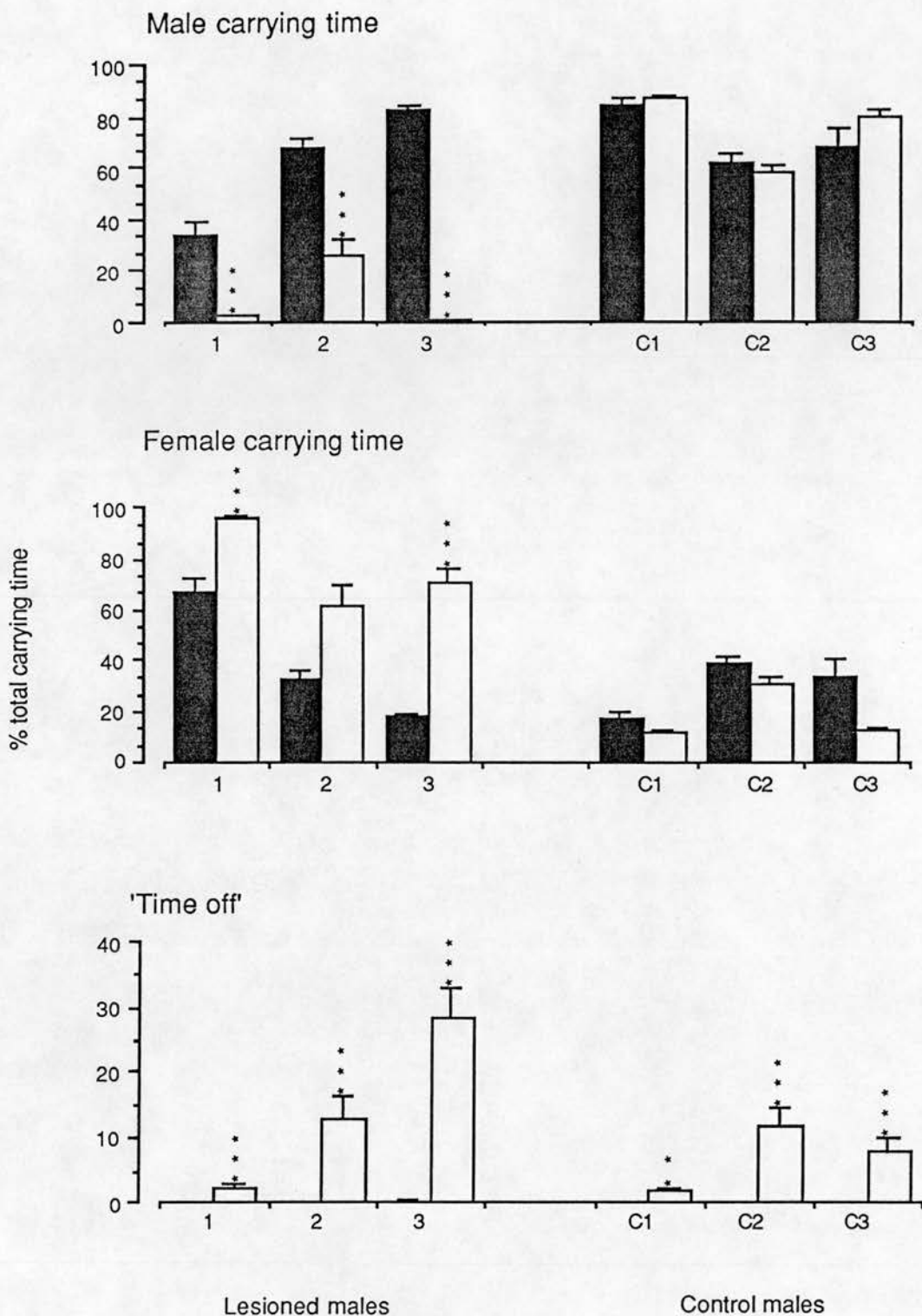


Fig. 6.3 Effects of POA-AH lesions (male nos. 1-3) or sham lesions (male nos. C1-C3) on infant carrying behaviour.

Histograms show mean \pm s.e.m. % total carrying time per day pre- (solid bars) and post-lesion (open bars). *** $P < 0.001$, Mann Whitney U-test (within-animal comparisons).

Fig. 6.4 shows the longitudinal data for the 6 groups. It can be seen that the decline in male carrying behaviour occurred abruptly on the day following surgery. Of equal interest is the recovery of carrying behaviour exhibited by male no. 2 on Day 21. This recovery occurred as rapidly as the initial deficit, and continued until the end of the study. The slight decline seen towards the end of the 30 days in this male was almost entirely due to an increase in transfers from the adults to the cage initiated by the infants as they gained independence during the second part of the study and is paralleled by the 3 control males - see Figs. 6.3 and 6.4.

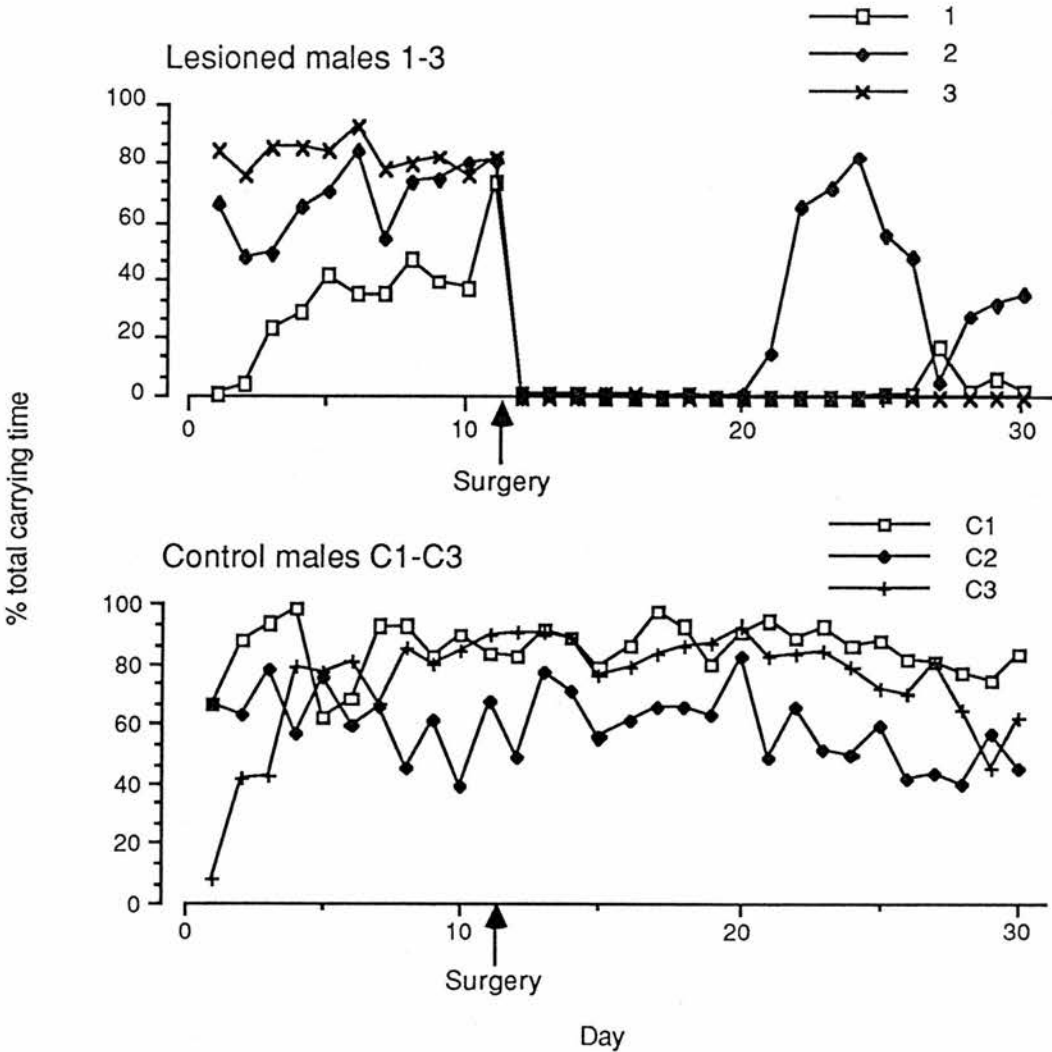


Fig. 6.4 Longitudinal carrying behaviour data for the lesioned males (nos. 1-3) and the control males (C1-C3).

Recovery from the lesion with respect to carrying behaviour also occurred in the case of male no. 3 though, in this instance, it was delayed until the group was removed from the video room and returned to the colony. For this reason, it was decided to follow this pair through the subsequent postpartum period to ensure that recovery was complete. During this period, the male's carrying behaviour occurred at similar levels to his pre-operative scores ($92.53 \pm 2.15\%$ of total time during the second postpartum period compared to $82.1 \pm 1.55\%$ during the pre-operative period), indicating that the recovery seen at the termination of the original study period was total and permanent.

It is interesting to note that the 2 males that showed recovery from the effects of these lesions on paternal behaviour had received the smallest lesions (see Fig. 6.2).

6.3.3 Effects on transfers

This decrease in carrying behaviour appears to be due to an active avoidance of transfers by the lesioned males. This becomes clear when the number of infant-initiated transfers refused by the males are taken into account. This measure is slightly complicated by the fact that during the pre-operative phase, very few infant-initiated transfers were seen in any of the groups, but there is a clear difference in the post-operative behaviour between the lesioned males and the sham lesioned controls in that the lesioned males refused a far greater percentage of these attempts than did the control males: male no. 1 refused 0% of 3 transfer attempts pre-operatively and 71% of 63 attempts post-operatively; male no. 2 refused 0% of 3 pre-operatively and 94% of 32 post-operatively (this male showed recovery on Day 21); and male no. 3 refused 8% of 26 pre-operatively and 81% of 62 post-operatively. In comparison, the increases in refusals from

the control males were much smaller: male no. C1 refused 0% of 5 transfer attempts pre-operatively and 4% of 56 attempts post-operatively; male no. C2 refused 0% of 6 pre-operatively and 54% of 149 post-operatively; and male no. C3 refused 0% of 7 pre-operatively and 15% of 145 post-operatively.

Another indication of the active avoidance of carrying the infants by the males is evidenced by the number of bouts of male carrying behaviour that were terminated by the males with dislodge behaviour. Again, the results are not totally clear-cut as the control males show an increase in dislodge behaviour during the post-operative period as the adults begin to force independence on the infants, but the percentage of bouts of their own carrying behaviour that are terminated by the lesioned males is clearly greater than those by the control males. This increase is also coincident with surgery in the lesioned males - see Fig. 6.5. When the actual percentages are considered, the fact that the lesioned males terminate a far greater percentage of carrying bouts than the control males post-operatively is clear: male no.1 terminated 8.2% of 61 bouts pre-operatively and 80% of 45 bouts post-operatively; male no. 2 terminated 0% of 74 pre-operatively and 29% of 135 post-operatively (this male showed recovery on Day 21); and male no. 3 terminated 14% of 193 carrying bouts pre-operatively compared with 88% of 60 post-operatively. In comparison, the increases in terminations by the control males post-operatively were smaller; male no. C1 terminated 0% of 42 bouts pre-operatively and 10% of 146 post-operatively, male no. C2 terminated 9% of 82 pre-operatively and 42% of 266 post-operatively, and male no. C3 went from 0% of 93 to 28% of 291.

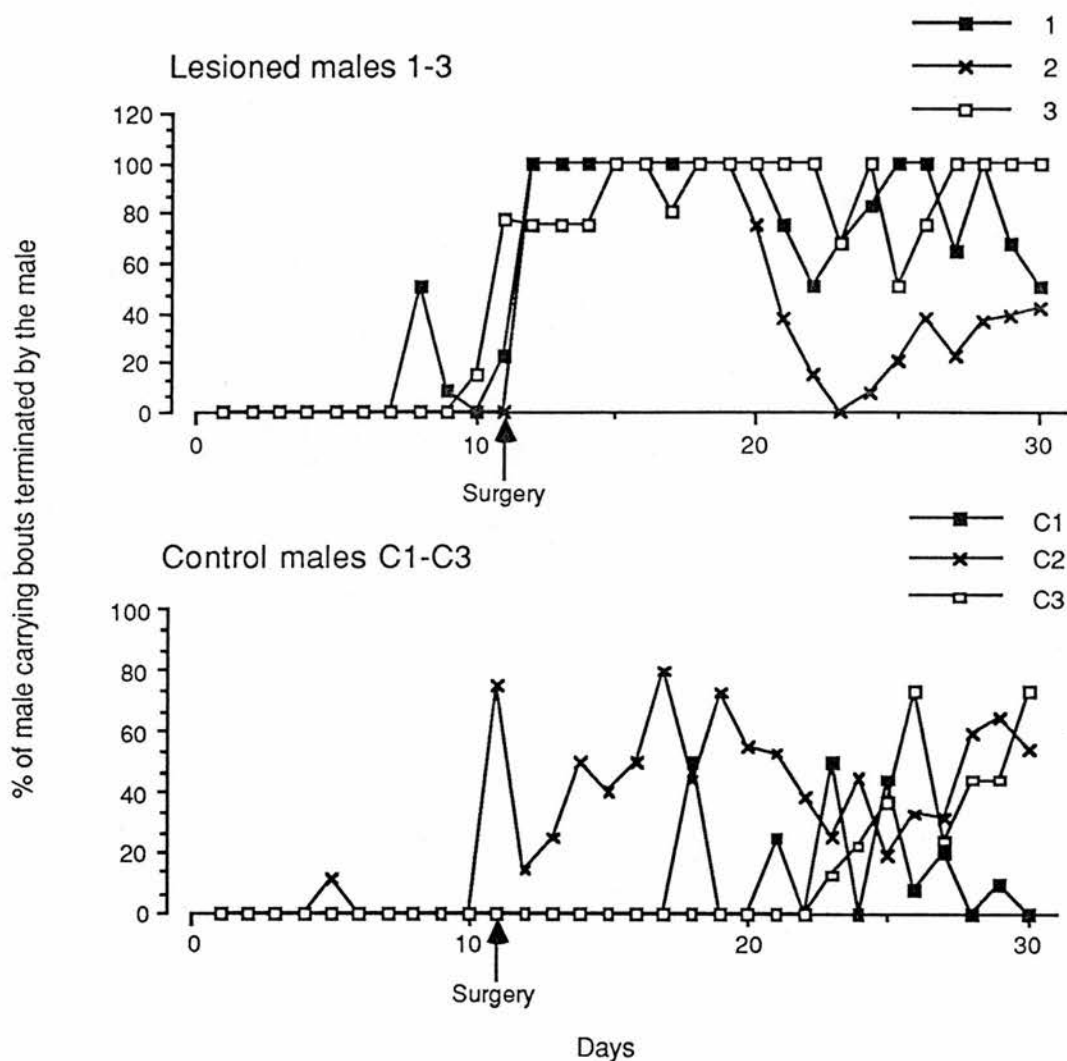


Fig. 6.5 The % of male carrying behaviour bouts terminated by the male himself.

From these results, it is clear that the lesioned males actively avoid carrying the infants by refusing transfers, or by dislodging the infants shortly after a successful transfer initiated by the infants themselves or by the females.

6.3.4 Effects on grooming behaviour

Fig. 6.6 indicates that this active avoidance of the infants is limited only to carrying behaviour as both allogrooming and anogenital grooming of the infants continues at or above pre-operative levels. In fact, in the case of

allogrooming, Mann-Whitney U-tests within animals indicated significant increases for male no. 1 (302.73 \pm 87.04 to 1254.36 \pm 133.36 secs. per day, $n_1=11$, $n_2=19$, $U=19$, $P<0.002$) and male no. 3 (502.18 \pm 191.22 to 1014.63 \pm 134.85, $n_1=11$, $n_2=19$, $U=48$, $P<0.02$). There were no significant alterations in the grooming behaviour of the control males towards their infants.

The 3 lesioned males showed no significant changes in allogrooming directed towards their female partners, though 2 of the control males (nos. C1 and C3) showed significant increases in this behaviour during the post-operative period - see Fig. 6.6. When the females' allogrooming of the males is considered, again there are no significant changes post-operatively - see Fig. 6.7. It appears that there may have been a tendency towards more of the female's allogrooming bouts being initiated by grooming invitations from the lesioned males during the post-operative period, but due to the small number of female allogrooming bouts in some cases, no statistical analysis could be carried out. However, male no. 1 initiated 0% of 14 allogrooming bouts pre-operatively and 17% of 35 post-operatively. In the case of male no. 2, this increase went from 20% of 5 bouts pre-operatively to 94% of 18 bouts post-operatively, and male no. 3 showed a rise from 13% of 151 pre-operatively to 52% of 158 post-operatively. The results from the control males do not show the same increases; male no. C1 initiated 0% of 33 female allogrooming bouts pre-operatively and 8% of 210 post-operatively, male no. C2's female only allogroomed him twice during the pre-operative period, once in response to a grooming invitation, and the male initiated 24% of 29 grooming bouts post-operatively. For the final control male no. C3, the pre-operative figure was 0% of 23 and the post-operative figure was 9% of 23.

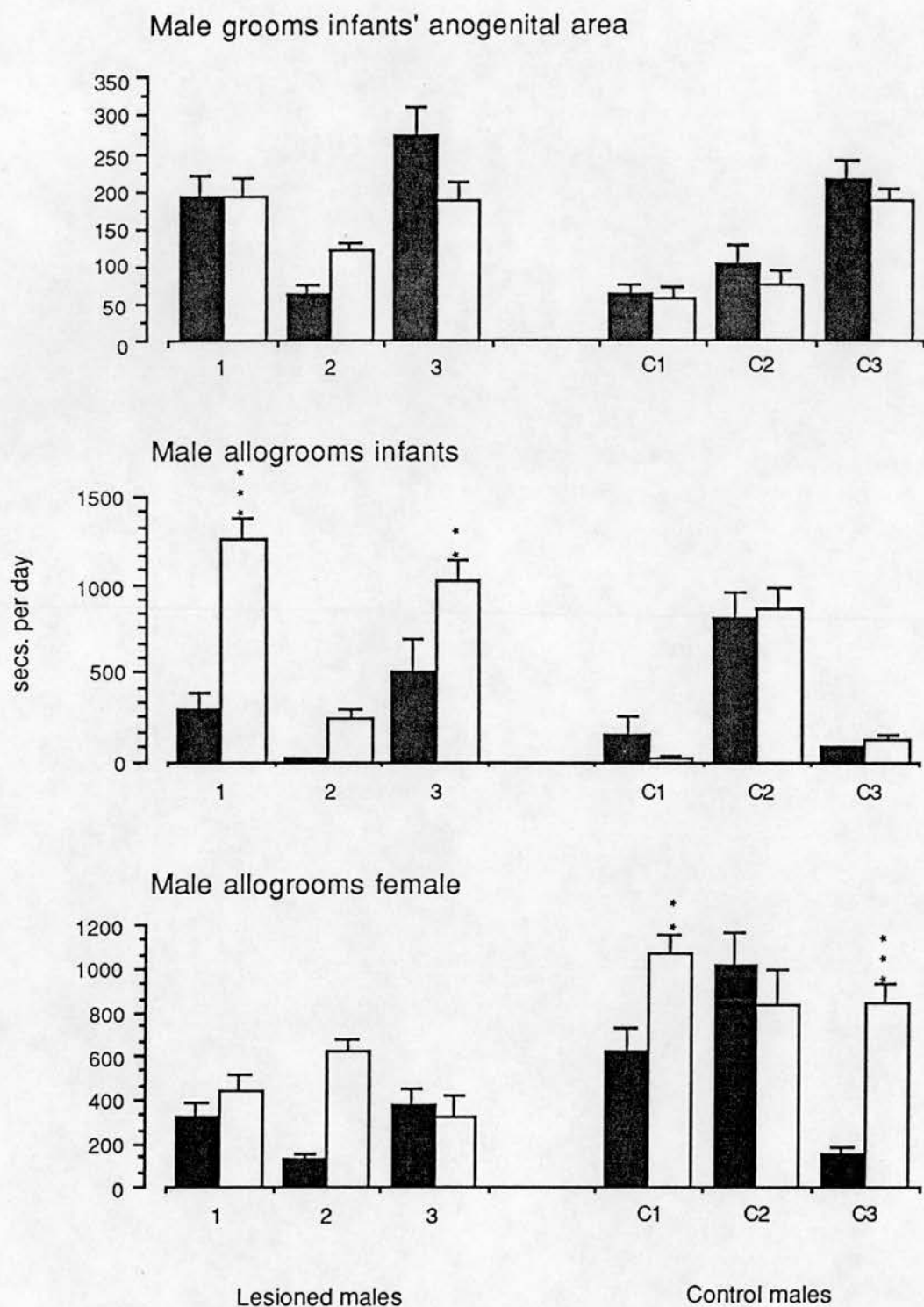


Fig. 6.6 Effects of POA-AH lesions (male nos. 1-3) or sham lesions (male nos. C1-C3) on male anogenital grooming and allogrooming.

Histograms show mean \pm s.e.m. secs. per day for each male pre- (solid bars) and post-lesion (open bars). *** $P < 0.002$, ** $P < 0.02$ Mann-Whitney U-test (within-animal comparisons).

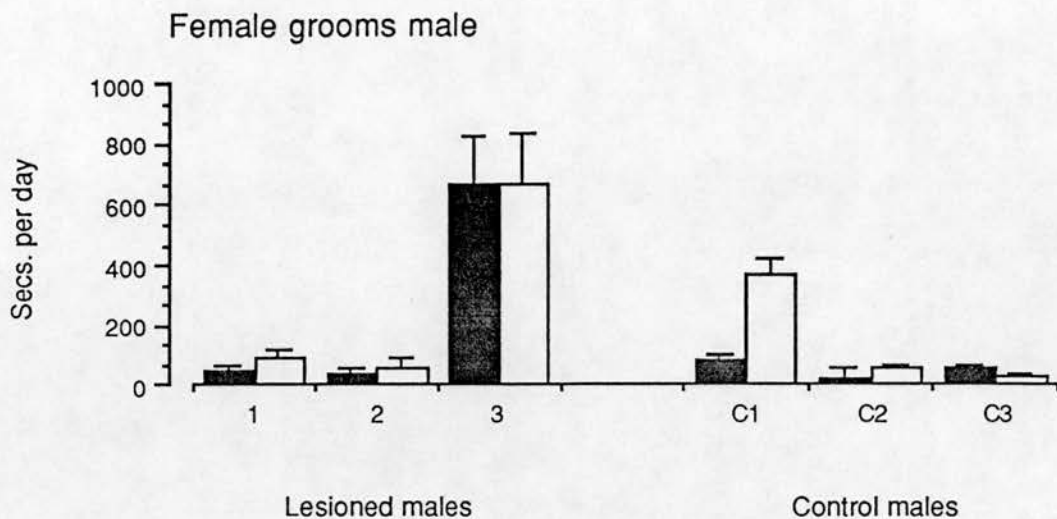


Fig. 6.7 Effects on the allogrooming of the female partners of POA-AH lesioned (nos. 1-3) or sham lesioned (nos. C1-C3) males.

Histograms show mean \pm s.e.m. secs. per day for each female pre- (solid bars) and post-lesion (open bars).

6.3.5 Effects on developmental markers

Several markers of the development of the infants were used to indicate the level at which the females compensated for the lack of paternal care. Fig. 6.8 shows the day on which the infants first spent 'time off' their parents, the day after which 'time off' occurred on each subsequent day and the day on which the infants were first seen to be eating solid food. The fact that these events occurred at similar times in both the lesioned males' and the control males' groups indicates that the female partners totally compensated for the lack of paternal care, and that the development of the infants was not delayed. This is further evidenced by the fact that all the infants had average body weights (averages calculated from 10 years of colony records) for their age during infancy - see Fig. 6.8. Unfortunately, the weights were not taken regularly during this period, though all the infants were healthy throughout the study period and developed into perfectly normal youngsters.

No.	1st 'time off'	Regular 'time off'	1st solid food	Age at weighing	Optimal (g)	Actual (g)
1	16	16	26	3	27-30	32.5 /32.2
2	12	12	24	40	50-80	91.0/89.6
3	5	10	22	30	44-64	68.6/67.4
C1	10	19	29	40	50-80	55.6/57.7
C2	2	14	22	6	28-36	33.0/30.0
C3	12	15	22	30	44-64	73.7/75.6

Fig. 6.8 Effects of a lack of paternal behaviour on developmental markers in twin infant marmosets.

Numbers in table refer to days after parturition and weight in grams. Optimal = weight range of infants in the colony through the last 10 years at the given age. Actual = individual weights of twin infants in the present study at the given age.

6.3.6 Effects on sexual behaviour

The effects of lesions on the postpartum sexual activity of these males are shown in Fig. 6.9. The control males show the typical postpartum pattern of sexual behaviour in this primate species. An initial period of little or no sexual activity is followed by an increase in the number of mounts and ejaculations, presumably coincident with the female's postpartum ovulation. This increased level of copulatory behaviour is continued until the end of the 30 day study period even though the female partners of male nos. C2 and C3 conceived following this postpartum ovulation and were therefore in the early stages of pregnancy by Day 30.

When the mating pattern of the lesioned males is considered, the initial pattern of little sexual activity followed by an increase in mounts and ejaculations is seen as in the controls. However, there is an abrupt decline in the number of mounts per day following surgery, and no intromissions or ejaculations were seen for any of the lesioned males during the post-

operative period. Therefore no data on intromission or ejaculation latencies following these lesions was available. This lack of sexual activity is particularly interesting in the case of male no. 2 which showed a recovery of parental behaviour on Day 21 though there was no parallel recovery in copulatory behaviour.

The mating activity that occurred prior to surgery did so with little or no precopulatory behaviour from either the male or the female. Following POA-AH lesions, the males were not seen to show any sexual interest in the females, in the form of anogenital sniffing or mount attempts. It therefore appeared that the males were suffering from a lack of interest in sexual activity. However, the lack of precopulatory behaviour observed between the normal pairs in this situation might suggest that these behaviours play a less important role in the initiation of sexual activity between permanently paired animals.

Female mount initiations, refusals and terminations occurred at low levels throughout the 30 day postpartum period so that no statistical analysis of the data was possible, though the largest number of refusals and terminations occurred in the first 20 days postpartum - see Fig. 6.10. This figure also shows that the first mount following parturition in every case was initiated by the male though this may not always have resulted in an ejaculation. The timing of resumption of sexual activity following parturition varied from Day 2-8, though the first ejaculatory mount did not occur until Day 7 or 8 in every pair.

The female partner of male no. 1 did not conceive whilst she was paired with this male (up to 46 days postpartum) though male no. 2's partner conceived (during the postpartum oestrus) and male no. 3 successfully impregnated his female (during the second oestrus cycle postpartum). These pregnancies were calculated from the subsequent interbirth interval.

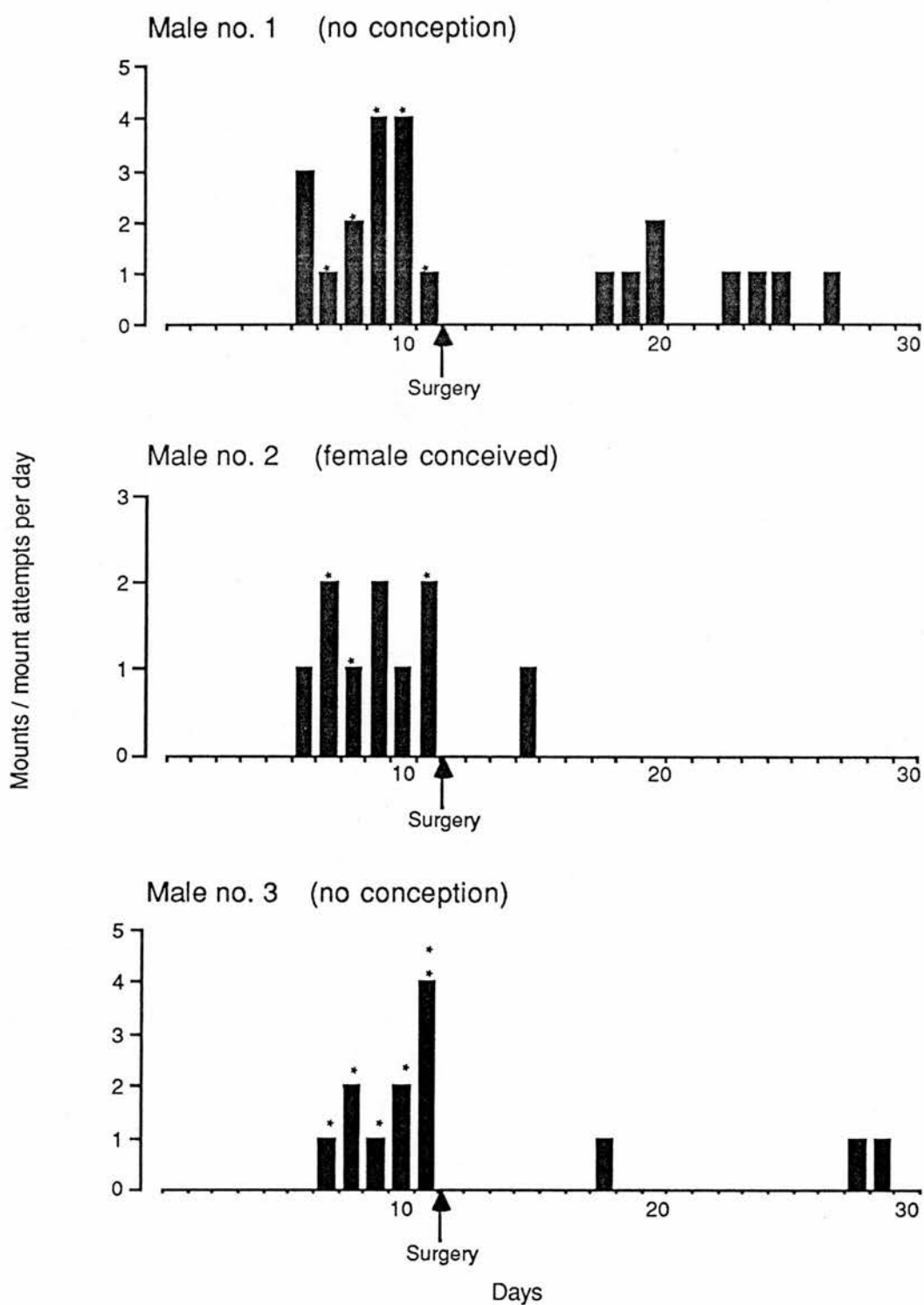


Fig. 6.9a Effects of POA-AH lesions on sexual behaviour during the postpartum period.

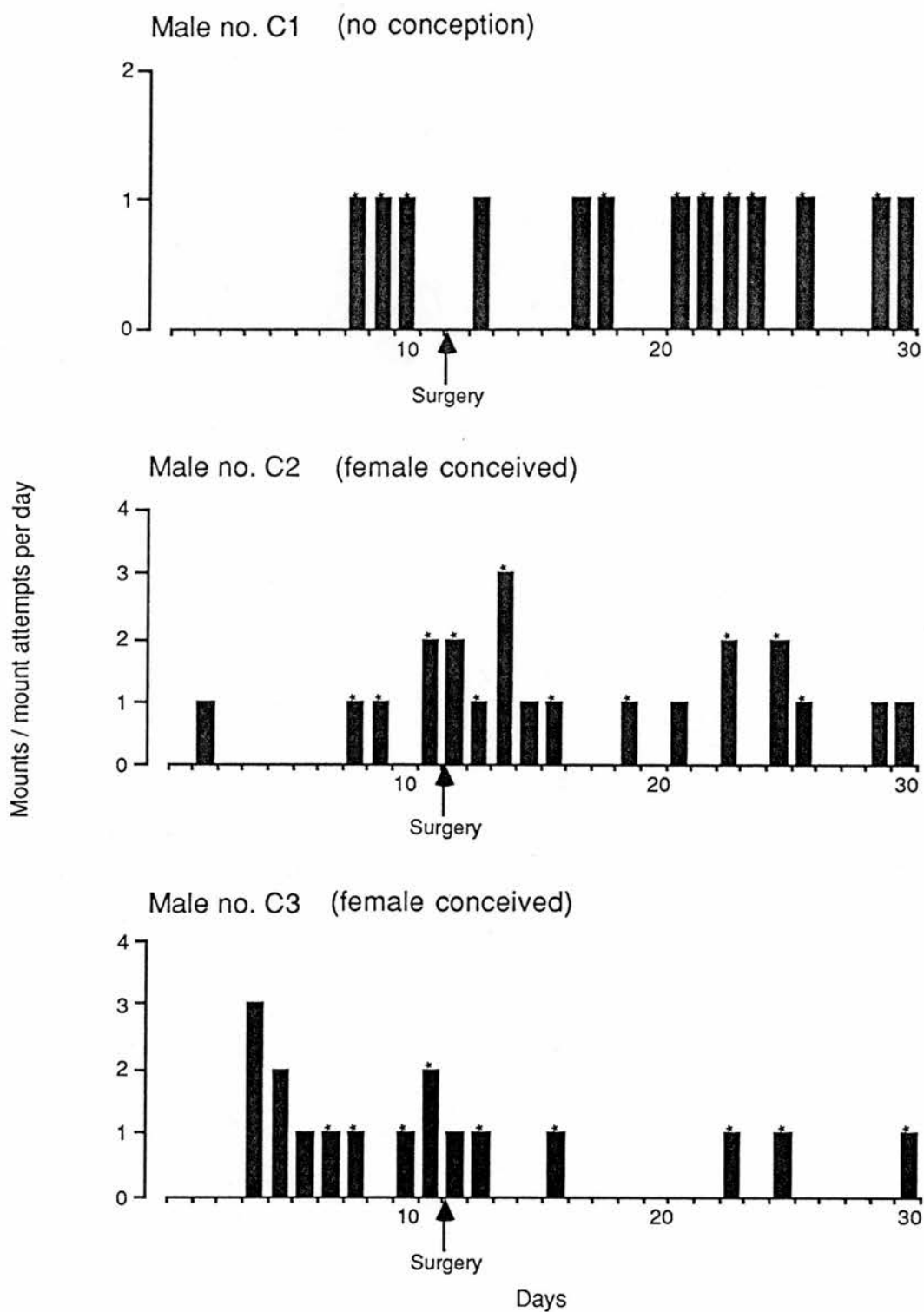


Fig. 6.9b Effects of sham lesions on sexual behaviour during the postpartum period.

Histograms show mounts and mount attempts per day for each male.
 * = ejaculation.

No.	Onset of sex	1st ejaculation	% mounts initiated by female			% mounts refused by female			% mounts terminated by female		
			1-10	11-20	21-30	1-10	11-20	21-30	1-10	11-20	21-30
1	6 (m)	7 (m)	0 (10)	0 (9)	0 (4)	30.0 (10)	22.2 (9)	0 (4)	0 (10)	0 (9)	0 (4)
2	6 (m)	7 (m)	0 (7)	33.3 (3)	0 (0)	14.3 (7)	0 (3)	0 (0)	14.3 (7)	0 (3)	0 (0)
3	7 (m)	7 (m)	0 (6)	20.0 (5)	0 (2)	16.7 (6)	40.0 (5)	0 (2)	0 (6)	0 (5)	0 (2)
C1	8 (m)	8 (m)	0 (3)	0 (3)	42.9 (7)	0 (3)	0 (3)	0 (7)	0 (3)	33.3 (3)	0 (7)
C2	2 (m)	8 (m)	0 (3)	0 (11)	0 (8)	0 (3)	0 (11)	0 (8)	33.3 (3)	27.3 (11)	0 (8)
C3	4 (m)	7 (m)	11.1 (9)	20.0 (5)	0 (3)	0 (3)	0 (5)	0 (3)	11.1 (9)	0 (5)	0 (3)

Fig. 6.10 Effects of POA-AH lesions (male nos. 1-3) or sham lesions (male nos. C1-C3) upon the postpartum sexual behaviour of pairs of common marmosets.

Figures for the onset of sexual behaviour (1st mount or attempted mount) and the 1st ejaculation refer to the postpartum day on which these occurred. Letters in parentheses refer to the initiator of the mount (m = male). The % of total mounts and mount attempts that the females initiated, refused or terminated for postpartum days 1-10, 11-20 and 21-30 are also shown. Figures in parentheses refer to the total number of mounts or mount attempts within each time period.

6.3.7 Effects on general activity levels

Fig. 6.11 shows the effects of the lesions on locomotor activity, scent marking and eating bouts during the first hour of each day. Drinking bouts occurred at such low frequencies both pre- and post-operatively that no statistical analysis was possible. It is evident that there was no general decline in activity levels in the lesioned or sham lesioned males but there was a tendency towards increased eating bouts in the case of the lesioned males though this was limited to the immediate post-operative phase - see Fig. 6.12. There was a significant decline in locomotor activity in the case of male no. 2, though this was paralleled by a similar decline in control male no. C2.

6.3.8 Effects on androgen-dependent morphology

Blood samples were not collected from the males during the study period to avoid interfering with the behaviour that was being scored. However, at the termination of the study, the androgen-dependent penile spines and testes of these males were normal and spermatogenesis was still occurring. As one of the males successfully impregnated their female partners following surgery, hence proving that it was fertile and potent at this stage, it is unlikely that the behavioural effects observed were due to any disruption of the hypothalamic-pituitary axis.

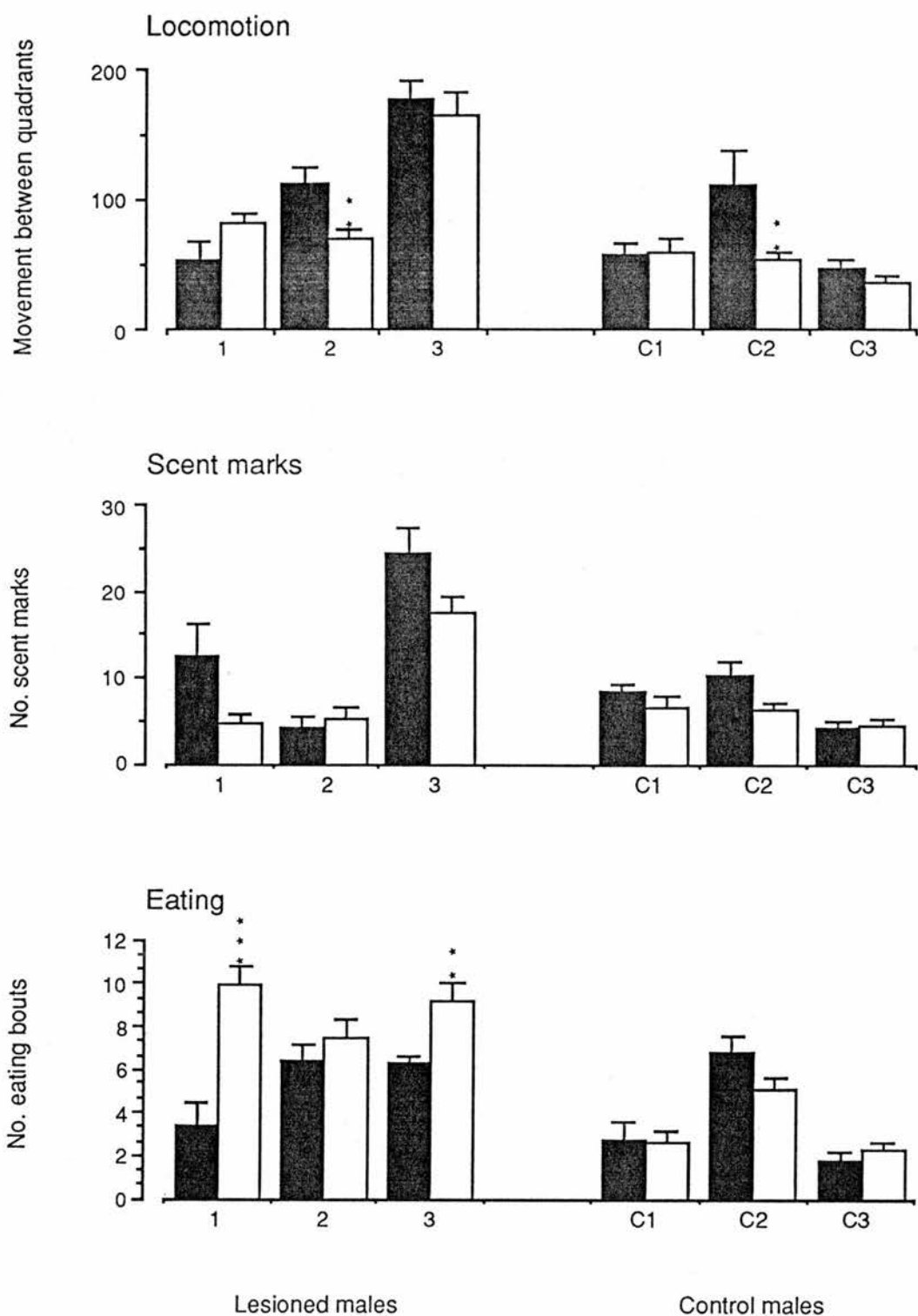


Fig. 6.11 Effects of POA-AH lesions (male nos. 1-3) or sham lesions (male nos. C1-C3) on general activity levels during the first hour of each day.

Histograms show mean \pm s.e.m. for each male pre- (solid bars) and post-lesion (open bars). *** $P < 0.002$, ** $P < 0.02$ Mann-Whitney U-test (within-animal comparisons).

6.3.9 Effects on general health

The males recovered rapidly from surgery and remained in good health until the conclusion of the study. There was a tendency towards hyperphagy immediately post-operatively, as evidenced by Fig. 6.12, and this resulted in increases in body weight in the 3 lesioned males - see Fig. 6.13.

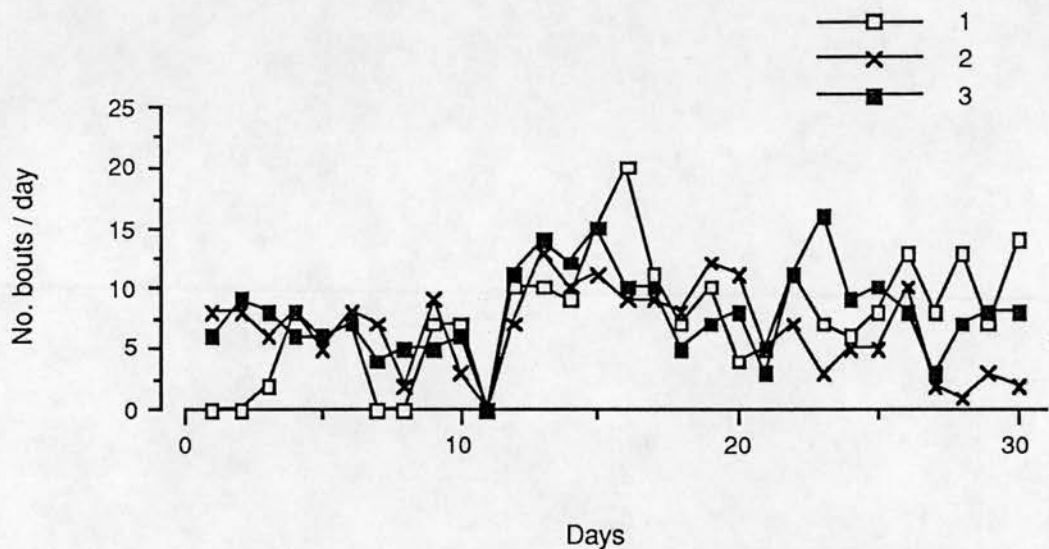


Fig. 6.12 Effects of POA-AH lesions (male nos. 1-3) on eating behaviour.

No.	Body weight (g)		
	Pre-operative	Post-operative	% increase
1	363	412	13.5
2	337	396	17.5
3	309	369	19.4
C1	311	313	0.6
C2	328	318	-3.0
C3	344	348	1.2

Fig. 6.13 Pre- and post-operative body weights (g) and % increase for lesioned males 1-3 and control males C1-C3.

In all cases, basal body temperatures fell within the normal range (101 - 103°F for 8 normal males) during the post-operative period.

6.4 Discussion

Due to the small number of animals used in this study, statistical analysis was limited. Therefore, all the conclusions drawn are based on within-animal effects and care must be taken in attaching too much significance to these results.

Only 7 pairs of animals were used in this study for two main reasons. The first was that only experienced breeding pairs were used which necessitated their removal from the breeding colony at the Unit. As this colony only contained 30 pairs, there were periods of time when no suitable animals were available for the study, thus prolonging the time course of the experiment. However, the major factor limiting animal numbers was the time taken to accurately scan the video tapes and to analyse the data. It had been anticipated that this would take 80-90 hours per group but, in reality, it took in excess of 150 hours per group thus limiting the amount of data collected during the 18 month study.

However, the behavioural changes witnessed during this novel experiment were extremely pronounced and occurred immediately following surgery, thus strongly indicating that they were a direct effect of the surgery and therefore worthy of discussion.

POA-AH lesions had profound effects on some aspects of the paternal behaviour of male common marmosets and also caused severe deficits in sexual behaviour.

Carrying of the infants was severely disrupted following these lesions in all

three cases, and this appeared to be due to active avoidance of carrying by these males - as evidenced by the increase in transfer refusals and the higher incidence of dislodge behaviour following a successful transfer - rather than by a passive failure to initiate carrying of the infants.

It is interesting that the deficits in carrying behaviour in two of the males were reversed after 1-3 weeks, and that these two males had received the smallest lesions. The recovery of male no. 3 on the return of the group to a colony room may have been due to the presence of a higher stimulus for parental care at this time. In the video room, the group was isolated from other animals and had very little human contact. However, on return to the colony, other animals were present in adjacent cages and there were more visits from humans. It may have been that this greater perceived threat towards the infants stimulated the recovery of paternal care at this stage. However, this male must have been predisposed towards recovery, as male no. 1 showed no such resumption of paternal behaviour when his group was returned to the colony.

The recovery of male no. 2 was of particular interest because of the way in which it occurred. The infants had attempted to initiate transfers onto the male at intervals since surgery, though these had mainly been rejected by the male. However, on Day 21, this male accepted a transfer attempt by one of the infants (having refused others earlier in the day) and, from then on, recovery was complete.

Another important factor to consider when discussing data on male carrying behaviour is the great variation between animals - which necessitated each male acting as his own control and surgery being performed during the postpartum period. It would appear that, as well as the quantity of paternal care, the time of onset is also variable between groups - see Fig. 6.4. This seems to be due to the reluctance of some females to allow the males

access to the infants during the immediate postpartum period.

Clearly the deficits in paternal behaviour are limited to carrying of the infants, as both anogenital grooming and allogrooming of the youngsters are unaffected or increased by the surgery. It is interesting that, in Experiment 1, grooming behaviour between adults was also increased following hypothalamic lesions, and it was argued that this could be an example of displacement activity - see Discussion, Chapter 4. It is possible that the increases in allogrooming towards the infants occurred for the same reasons though, in this experiment, no similar effects were seen on male allogrooming directed towards the female despite profound deficits in sexual activity. One explanation of this might be that, in a permanent group situation in an isolated room, the motivation to strengthen pair bonding by increased social interactions may not be as great as during infrequent pair tests in a room containing other animals. Continuing this argument, by Day 11 postpartum the males may not have formed a strong bond with their offspring (possibly achieved by the close proximity during carrying), so that the motivation to do so may stimulate increased grooming activity when carrying behaviour ceases.

In the experiments described in Chapter 4, displacement activities in the form of eating and drinking were impossible due to the absence of food or water during pair tests. However, in the present study, the males had access to both food and water throughout the study period. Therefore, if displacement activity similar to that seen by Hansen and Drake at Hagelsrum (1984) in rats was occurring, it would have been apparent under these conditions. During the course of the 30 days, males were never seen to eat or drink following an abortive mount or following an interaction with the infants in a way which suggested to the observer that the behaviour was occurring in response to thwarted sexual or paternal motivation. It therefore

appears that, under these circumstances at least, male marmosets do not exhibit the same classical displacement activities as those expressed by male rats following MPOA lesions.

It is interesting that in the present study and in the experiments described in Chapter 4, almost all the lesioned males showed increases in body weight post-operatively. It is clear from this evidence that the animals do experience increased ingestion rates following hypothalamic lesions, at least transiently - see Fig. 6.12, and it is possible that the effects seen by Hansen and Drake and Hagelsrum were partly due to a direct effect on ingestive behaviour and were not purely examples of displacement activity.

In rat studies it has been argued that the active or oral aspects of maternal behaviour (nest building and pup retrieval) are preferentially abolished by MPOA lesions or severance of its dorsolateral connections, whilst the more passive crouching and nursing behaviours are less affected (Terkel et al., 1979; Jacobsen et al., 1980). In the present study, active and oral components, i.e. grooming behaviour, are spared by these lesions whilst transfers and carrying behaviour (the latter being more passive in nature) are severely disrupted. Thus, there appears to be a contradiction between the present study and earlier literature, though the justification for comparing maternal and paternal behaviour and rodents and primates directly is questionable.

Another way of interpreting the data is to consider that carrying behaviour (and its associated transfers) is the only purely paternal behaviour shown by marmosets, i.e. males only display this behaviour towards infants, whilst anogenital grooming and allogrooming also occur between adults. This raises the interesting possibility that the behavioural deficits following these lesions are limited solely to paternal behaviour and sexual activity. A possible explanation of the fact that POA-AH lesions appear to disrupt both

paternal behaviour and masculine sexual behaviour is given in Chapter 8.

The effects of the lesions on paternal behaviour were not due to a general social withdrawal, or to withdrawal from the infants in particular, as evidenced by the data on grooming behaviour. It was also clear during observations that the males did not attempt to avoid physical contact with the female - for example, the two adults continued to sleep huddled together during the dark period. If the males were hypersensitive to touch, one would expect this huddling behaviour to decrease and to see a decline in female allogrooming and male grooming invitations. As this did not happen, it is unlikely that the deficits in carrying behaviour seen during the post-operative period are due to any abnormality in the sense of touch.

It would also appear that the lesions did not result in a general decrease in activity levels, which may then have led to a decline in carrying behaviour. The results from the data on activity levels during the first hour of each day indicate that, in general, the males continued to be highly active throughout the post-operative period.

Another possibility is that the deficits seen are due to the effects of these lesions upon the hormonal status of the males. It was decided that to take blood samples from the males throughout the course of the experiment would cause the pairs unnecessary stress which may have led to lower levels of parental care, or even to rejection of the infants. Therefore the hormonal status of these males is not known, though there are some indirect measures which give an indication of this. From the previous lesion study (see Chapter 4), it is clear that thermal lesions within the POA-AH do not influence plasma testosterone levels. Indeed, the males in the present study all had normal androgen-dependent penile spines and testes at the conclusion of the experiment. Also, one of the males (male no. 2) successfully impregnated his female partner following surgery indicating that

this male was still fertile and potent. In the light of this evidence, it is unlikely that the effects seen on paternal behaviour are due to changes in plasma testosterone levels.

Plasma prolactin levels rise whilst male common marmosets are carrying infants (Dixon & George, 1982), so it is possible that the males in the present study had low basal levels of prolactin which then led to a deficit in carrying behaviour. However, Dixon and George found that levels only rise whilst the males are actually carrying the infants and then fall sharply back to normal levels once the infants have been removed. It is therefore unlikely that low basal levels of plasma prolactin in these males would influence their behaviour. As the males carried the infants for such short periods of time following surgery, it is unlikely that their plasma prolactin levels would have risen before the infants were dislodged. The function of the rise in plasma prolactin levels which occurs in normal males during carrying behaviour is unknown at present.

Hormones are clearly important in the control of maternal behaviour in all species studied to date - see Chapter 2 - though their role in paternal behaviour is less obvious. Males do not undergo the hormonal changes associated with pregnancy and parturition which are generally considered to 'prime' the female for the subsequent onset of maternal care. Furthermore, all individuals in a group of common marmosets will carry infants, even immature animals whose hormone profiles are profoundly different to those of their adult parents (Tardif et al., 1986). This indicates that the hormonal status of the male is unlikely to influence the onset and maintenance of paternal care in the common marmoset.

These lesions also caused marked decreases in sexual behaviour. Although some mounting was seen post-operatively for all three lesioned males, no intromissions or ejaculations occurred, in comparison with the

sham lesioned males which continued to exhibit ejaculatory mounts up to Day 30 postpartum, despite conception following the postpartum ovulation in two of the three females (male nos. C2 and C3). It is interesting that the third male (no. C1) continued to mount and to ejaculate throughout the 30 days as, in a previous study (Dixson & Lunn, 1987), a clear difference was observed between conception and non-conception postpartum cycles. In the former, sexual activity was found to continue through the early stages of pregnancy whilst in the latter, sexual activity was highest during the 7 days around the females' postpartum ovulation though not limited to this period. Interestingly, the 6 females exhibited very little proceptive behaviour at any stage of the study. In particular, there was no evidence of higher levels of proceptivity coincident with the peak in mating activity which presumably reflects the periovulatory period. During 30 minute pair tests, all precopulatory and copulatory behaviours are accentuated and it is possible to observe changes in levels of proceptivity throughout the ovarian cycle, with the highest levels occurring during the periovulatory period (Kendrick & Dixson, 1983). Under more natural conditions when the male and female are housed together permanently, these highly ritualised behaviours appear infrequently. However, it is possible that under these conditions the females are employing more subtle invitational behaviours which were unobserved by the experimenter. In this context it is important to distinguish between the permanent groups studied in this investigation and groups in other contexts. Although, under the present conditions, proceptivity occurs only infrequently, it is possible that in groups that contain more than one male, in the wild when the individuals of a group are more widely spaced, or during the formation of a pair bond, female proceptive behaviours are extremely important cues.

It is clear that the 6 females are less sexually receptive during the immediate

postpartum period, which may partially explain the absence of sexual activity at this time. However, the males also rarely attempted to mount during this period suggesting that their female partners are unattractive at this time. In every case, the first postpartum mount or mount attempt was initiated by the male and, in all but one case, this first sexual activity was followed by an ejaculation on the same day, thus indicating that the females were receptive at the time at which the males chose to resume sexual activity.

When compared to the lesion placements in Experiment 1, the lesions in this study corresponded most closely to male nos. 7,8 and 9 (see Figs. 4.2 and 6.2). However, unlike male no. 8, the males in the present experiment all sustained damage within the AH, which may explain why these males showed severe deficits in sexual behaviour whilst male no. 8 did not.

The major criticism of this study is the fact that so few animals were studied. However, the effects on carrying behaviour and sexual activity were so profound and occurred so soon after surgery that their cause is unquestionable. There were significant fluctuations in other behaviours for both lesioned and sham lesioned males (for example, locomotion and grooming) which makes analysis of this data more difficult, and more subtle effects may have been masked by the small number of experimental animals.

CHAPTER 7

EXPERIMENT 4: OLFACTORY BULB SECTIONS AND MASCULINE SEXUAL AND PATERNAL BEHAVIOUR IN A PERMANENT GROUP SITUATION.

7.1 Introduction

The POA-AH receives major afferent connections from both the main and accessory olfactory systems via its connections with the amygdalae (Scalia & Winans, 1975). The importance of this pathway, and of olfactory inputs in particular, in the control of maternal behaviour in mammals has been outlined in Chapter 2 and it is clear from the experiment described in Chapter 6 that the POA-AH is also crucially important in the control of paternal behaviour in the common marmoset. It was therefore decided to investigate whether the neural inputs from the olfactory system influence paternal behaviour in the common marmoset in a similar way to the effects seen on maternal behaviour in a number of other mammalian species.

In an earlier study (Dixon & Lunn, 1987), it appeared that the postpartum rise in sexual activity seen in marmosets was not initiated by overt behavioural cues from the female. It is possible that there are olfactory changes coincident with the return to ovarian cyclicity that are detected by the males, thus leading to an increase in copulatory activity. This preliminary study was therefore designed to investigate the role of olfaction in the paternal behaviour of the common marmoset and in the patterning of sexual activity during the postpartum period in this species.

7.2 Materials and methods

7.2.1 Animal selection

As olfactory stimuli may influence the initiation of paternal behaviour in this species, it was deemed necessary to perform surgery prior to the birth of the infants. This experimental design precluded the use of both pre- and post-

operative data from the same post-partum period for each male. As there are large variations between males concerning the amount of paternal care displayed, it was decided to study each male through two consecutive postpartum periods and to compare their behaviour between these periods. Therefore, the control males from Experiment 3 (male nos. C1-C3) were chosen to be studied through the subsequent postpartum period with the same female partner.

Unfortunately, the female partner of male no. C1 had died in the intervening period so this male was excluded from the study leaving two males (nos. C2 and C3) in this preliminary investigation. The female partners of these two males both gave birth to twin offspring during the study.

Two normal males from the colony were chosen at random to act as controls during the olfactory discrimination tests.

7.2.2 Surgery

Olfactory bulb sections were carried out on the two males 2-3 weeks prior to the birth of the infants as described in Chapter 3. This technique was chosen to ensure the complete destruction of both the main and accessory olfactory systems as marmosets possess a vomeronasal organ. Recovery from surgery was rapid and uneventful in both cases.

7.2.3 Video recording

The animals used in this study were monitored using a time-lapse video recording system as outlined in Chapter 3. Behaviour was recorded at 4x normal speed and the tapes were run for the full 12 hours of the light period (0600h-1800h) each day.

7.2.4 Testing regime

The pairs were placed in the video cage 2-3 weeks prepartum to allow time for habituation to the changed environment before parturition. Tapes were recorded from this day onwards to ensure that the first day postpartum was captured on tape. In both cases, parturition occurred during the dark period. Each pair was recorded for 30 days postpartum and the first 2 hours of each day were scored for aspects of sexual, social and parental behaviour. For comparison with these data, the scores for the first postpartum period (i.e. the pre-operative period) were recalculated for the first 2 hours of each day. In addition, during the post-operative postpartum period, sexual behaviour was scored throughout the 12 hours of the light phase. In the case of the second male (no. C3), sexual behaviour was also scored for 12 hours per day for 6 days immediately prepartum.

Blood samples were taken from the two females to allow for monitoring of LH and progesterone levels at 2 day intervals from Day 6 until the conclusion of the experiment.

At the conclusion of the study, the two males also underwent a series of olfactory discrimination tests as described in Chapter 3.

7.2.5 Behaviours scored

The behaviours listed here are described in detail in Chapter 3.

During the first 2 hours of each day, the position of the twin offspring was recorded, i.e. on the female, on the male or during 'time off' both parents. For this reason, a total of 4 hours carrying time was recorded (2 hours for each of the twin offspring) each day. In addition, transfers, dislodge behaviour, re-establishment of contact, allogrooming and anogenital grooming of the infants and allogrooming between the two adults were also scored during this period. Mounts, intromissions, intromission latencies

(from the first mount), ejaculations and ejaculation latencies for the male and mount initiations, refusals and terminations by the female were scored throughout the 12 hour light period.

During the olfactory discrimination tests, approaches to and 'touches' of the two dishes of food were scored for each male.

7.3 Results

7.3.1 Olfactory discrimination tests

The results from the olfactory discrimination tests prove that the two males used in this experiment were totally anosmic. In each test, there were two food trays, one of which was normal and one of which had been contaminated with caproic acid. Each tray was wrapped in polythene which had holes made in it. Therefore, the males could see and smell both food trays but were unable to touch or taste the food. Male approaches to each tray and 'touches' (attempts to grab at a piece of food) were scored. Fig. 7.1 shows the results for the two anosmic males (male nos. C2 and C3) and two normal males which acted as controls (male nos. N1 and N2). Chi-squared tests reveal that the two normal males showed a significant preference for the normal food, as evidenced by approaches (male no. N1: $\chi^2 = 9.72$, $P < 0.005$; male no. N2: $\chi^2 = 8.56$, $P < 0.005$) and 'touches' (male no. N1: $\chi^2 = 19.0$, $P < 0.005$; male no. N2: $\chi^2 = 17.1$, $P < 0.005$), whilst the two anosmic males showed no preference for either the normal or the treated food over a series of 5 tests - see Fig. 7.1.

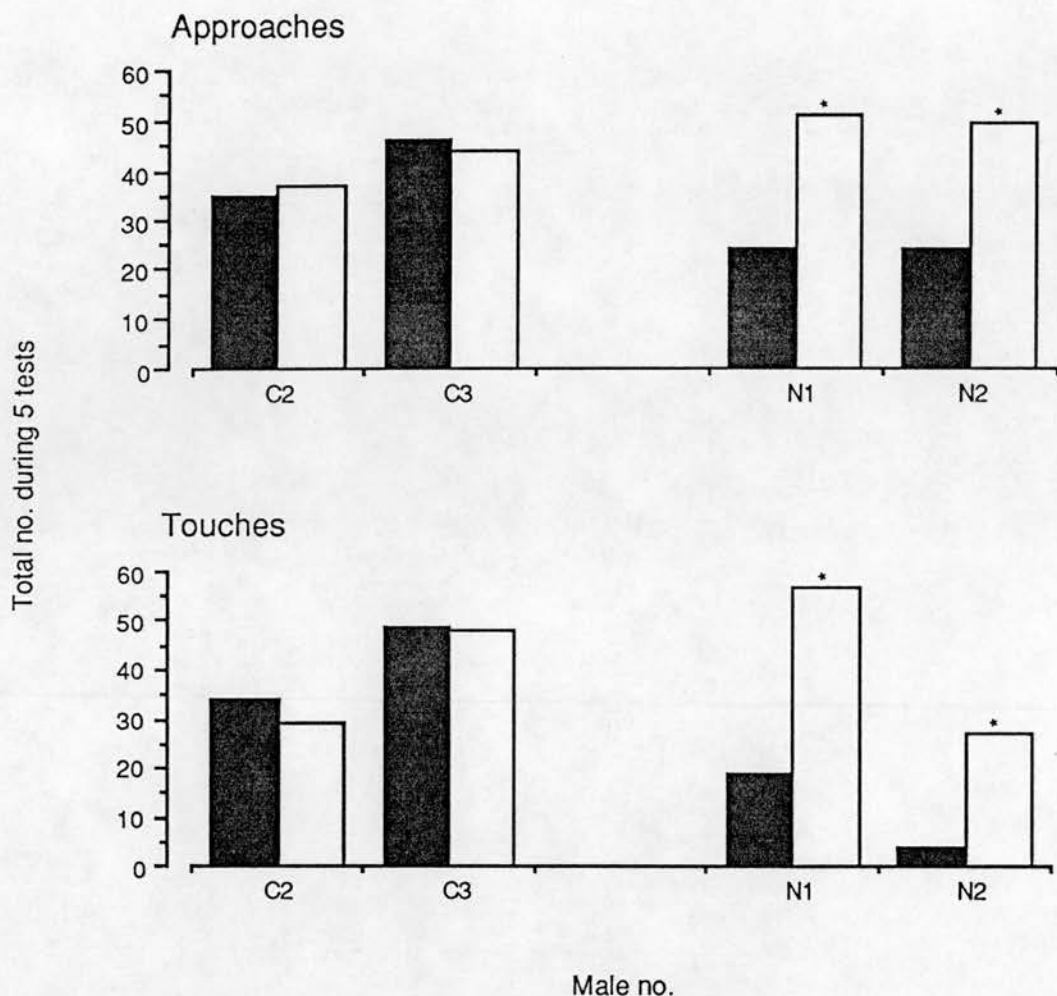


Fig. 7.1 Effects of olfactory bulb section on olfactory discrimination of caproic acid-contaminated food by male marmosets.

Male nos. C2 and C3 = anosmic. Male nos. N1 and N2 = normal controls. Closed bars = food contaminated with caproic acid. Open bars = normal food. * $P < 0.005$ Chi-squared test.

7.3.2 Effects on carrying time

The effects of olfactory bulb sections on carrying time are shown in Fig. 7.2. Male no. C2 showed no significant change in carrying behaviour following surgery though a Mann-Whitney U-test within-animals revealed a significant decline in the carrying behaviour of male no. C3 post-operatively (mean \pm s.e.m. from 73.85 ± 3.89 pre-operatively to 52.75 ± 2.69 post-operatively,

n=30, U=122, P<0.001) and this was paralleled by a significant increase in the carrying behaviour of the female in this group (from 23.22 +- 4.02 to 44.34 +- 2.97, n=30, U=136, P<0.001).

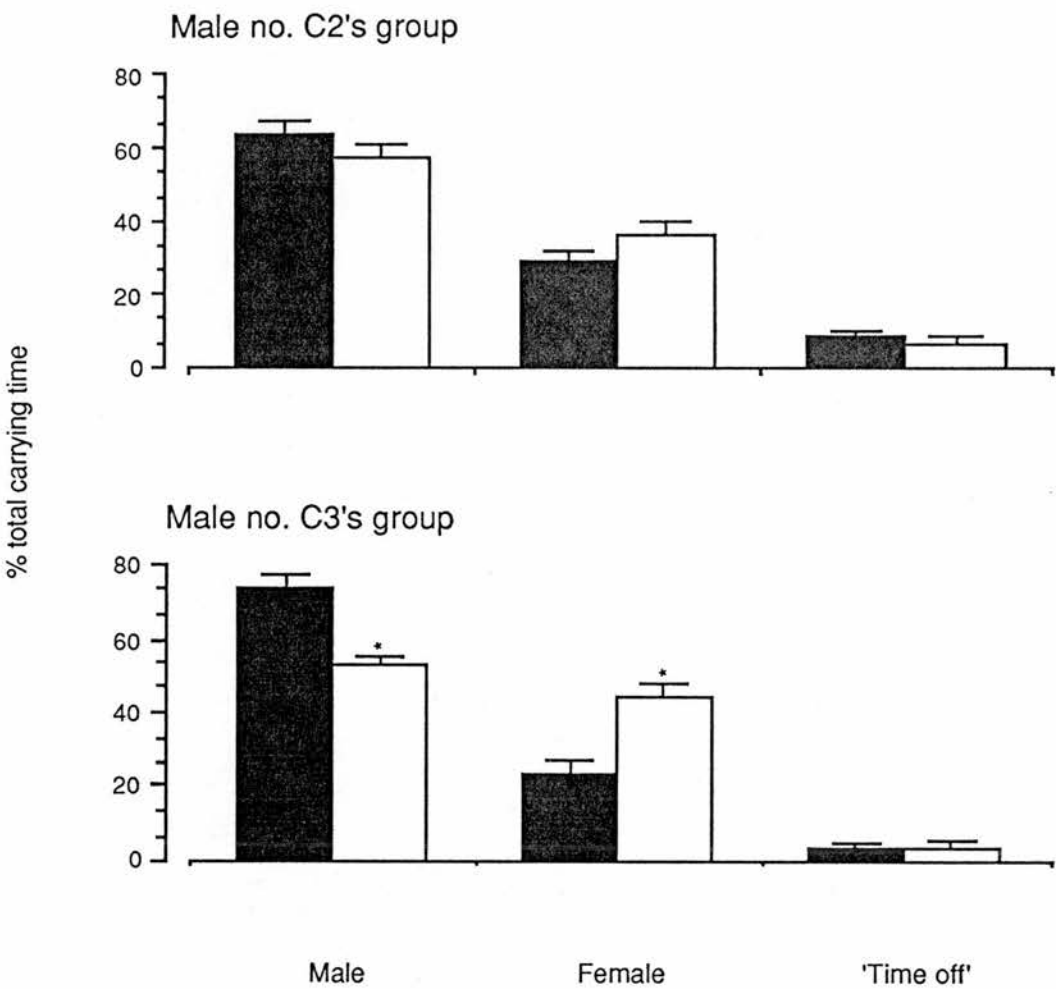


Fig. 7.2 Effects of olfactory bulb sections on the carrying behaviour of pairs of common marmosets.

Histograms show mean +- s.e.m. % of total time per day (n=30) for each group pre- (solid bars) and post-operatively (open bars). * P<0.001 Mann-Whitney U-test (within-animal comparisons).

7.3.3 Effects on transfers

When the number of infant-initiated transfers that the males refused and the number of carrying bouts terminated by the male are taken into account, it is

clear that the pre- and post-operative values for each male are similar, and that the decrease in carrying time seen for male no. C3 was not due to an active avoidance of the infants, but may simply reflect the natural variance seen in this behaviour between pregnancies (Ingram, 1977).

Male no. C2 refused 61.4% of 44 infant-initiated transfers during the pre-operative period and 47.6% of 21 during the post-operative period. The corresponding results for the male no. C3 were 43.2% of 37 transfers pre-operatively and 27.0% of 37 transfers post-operatively.

When the number of male carrying bouts that each male terminated with dislodge behaviour are considered, the pre- and post-operative values are again similar - male no. C2 terminated 35.0% of 143 bouts pre-operatively and 35.2% of 142 bouts post-operatively and the results for male no. C3 were 14.3% of 154 bouts pre-operatively and 19.4% of 139 bouts post-operatively.

7.3.4 Effects on grooming

Fig. 7.3 indicates that olfactory bulb sections influence the grooming behaviour of the males. Mann-Whitney U-tests within animals indicate significant declines in anogenital grooming of the infants by male no. C2 (mean \pm s.e.m. secs. from 31.2 \pm 5.94 to 15.97 \pm 3.68, $n=30$, $U=309$, $P<0.05$) and male no. C3 (from 101.5 \pm 11.66 to 73.33 \pm 13.74, $n=30$, $U=303$, $P<0.05$), allogrooming of the infants by male no. C2 (from 337.1 \pm 53.33 to 46.63 \pm 14.11, $n=30$, $U=136$, $P<0.001$) and male no. C3 (from 38.23 \pm 8.18 to 15.97 \pm 4.57, $n=30$, $U=305.5$, $P<0.05$), and allogrooming of the female by male no. C2 (from 369.0 \pm 57.09 to 49.13 \pm 18.69, $n=30$, $U=146$, $P<0.001$) and by male no. C3 (160.3 \pm 38.08 to 29.73 \pm 16.13, $n=30$, $U=157.5$, $P<0.001$).

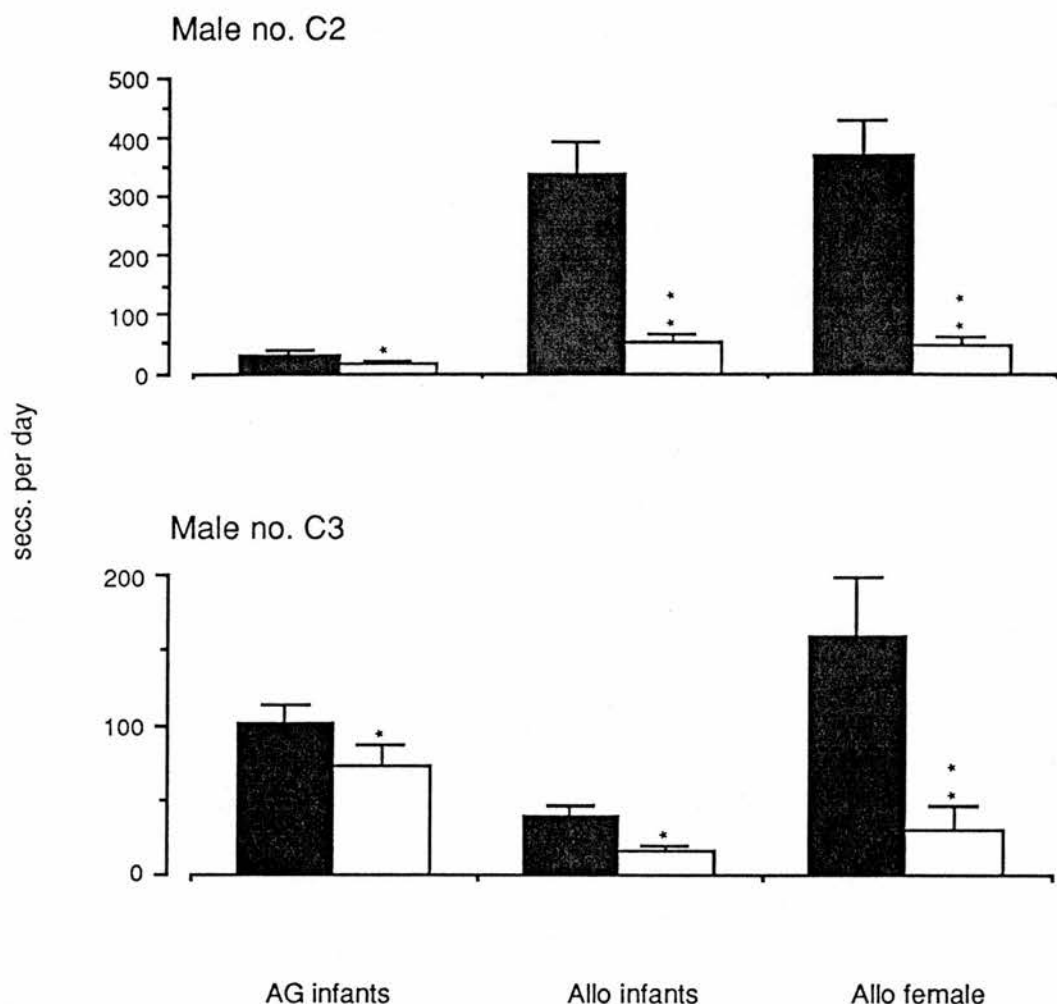


Fig. 7.3 Effects of olfactory bulb sections on male grooming behaviour.

Histograms show mean \pm s.e.m. secs. per day for each male pre- (solid bars) and post-operatively (open bars). AG infants = anogenital grooming of the infants, Allo infants = allogrooming of the infants and Allo female = allogrooming of the female by the male. * $P < 0.05$, ** $P < 0.001$, Mann-Whitney U-test (within-animal comparisons).

Fig. 7.4 illustrates that the allogrooming directed towards the males by their female partners was also decreased following the anosmia induced in the males in the case of male no. C2 (mean \pm s.e.m. 13.2 ± 4.65 pre-operatively compared to 0.87 ± 0.60 post-operatively) and male no. C3 (from 11.97 ± 5.00 to 0.00 ± 0.00 post-operatively). However, statistical analysis of these data was not possible due to the small number of allogrooming bouts performed by the females both pre- and post-operatively.

When the number of these grooming bouts that were initiated by the males are considered, the small number of pre- and post-operative female allogrooming bouts result in the significance of the figures being questionable. However, male no. C2 invited 2 out of 15 bouts pre-operatively and 1 out of 2 bouts post-operatively. Male no. C3 invited 1 out of 22 bouts during the pre-operative period but there were no female allogrooming bouts during the post-operative postpartum period.

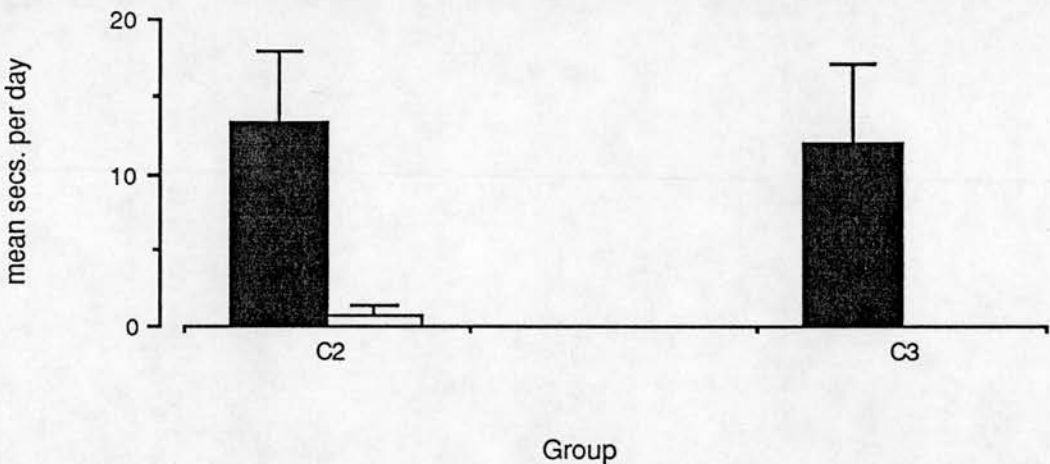


Fig. 7.4 Allogrooming behaviour of female marmosets paired with anosmic males.

Histograms show mean \pm s.e.m. secs. per day for each female pre- (solid bars) and post-operatively (open bars). No statistical analysis was possible due to the small n.

7.3.5 Effects on sexual behaviour

The pattern of sexual activity seen during the postpartum period of male no. C2 is shown in Fig. 7.5 and this pattern is clearly different to that seen in normal pairs during this time (see control male data in Chapter 6). Instead of an initial period of little or no sexual activity immediately postpartum, this male initiated a mount on Day 1 and achieved ejaculation on Day 2 postpartum. This level of sexual activity continued throughout the 30 day

monitoring period despite the fact that this female did not show a postpartum cycle during this time - see the progesterone profile in Fig. 7.5.

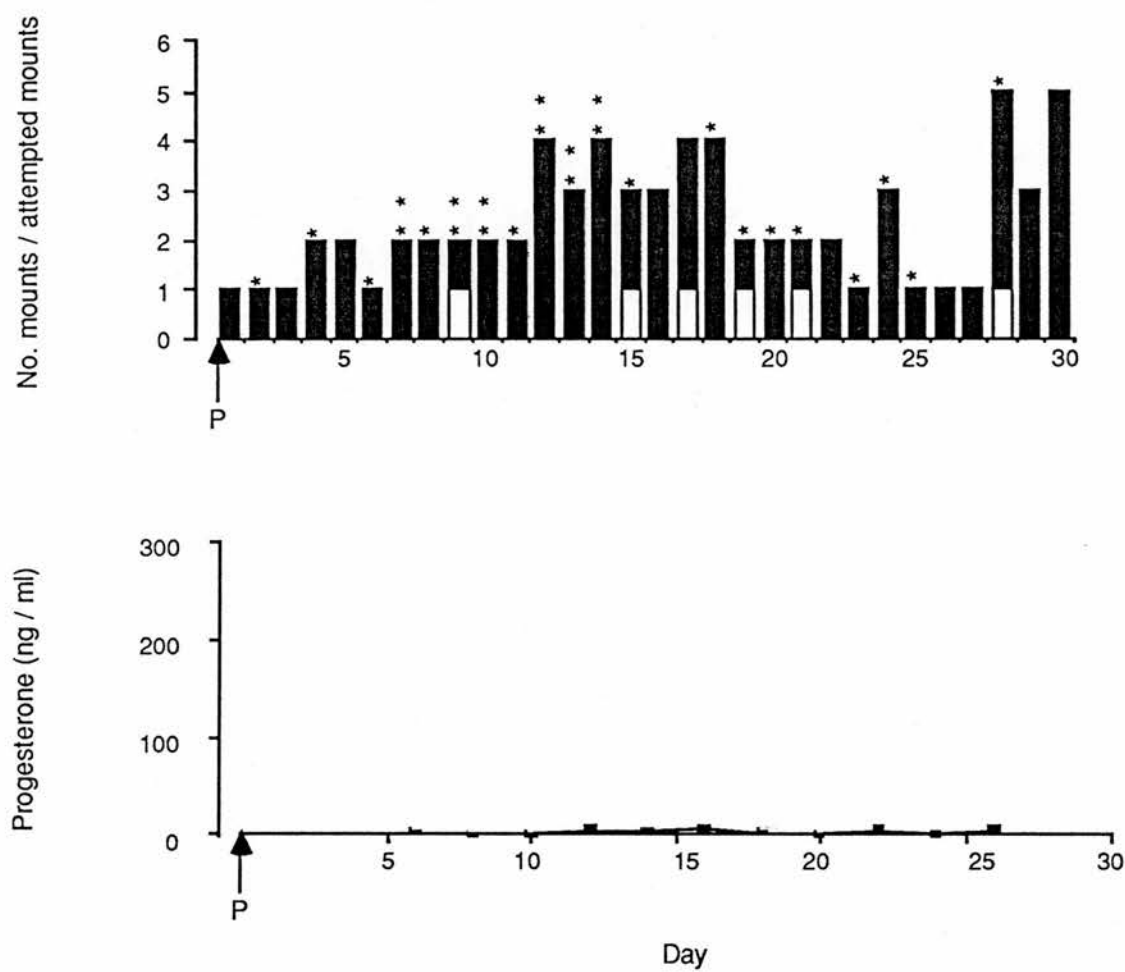


Fig. 7.5 Effects of an olfactory bulb section on the postpartum sexual behaviour between male no. C2 and his female partner in relation to the female's postpartum hormone profile.

The top histogram shows the number of mounts or attempted mounts during the 12 hour light period for each day. Male-initiated mounts = solid bars, female-initiated mounts = open bars. * = ejaculation. Plasma hormone levels are shown in ng/ml in the lower graph. P = day of parturition.

In view of the postpartum sexual activity between this anosmic male and his female partner, it was decided to also monitor the second group (no. C3)

during late pregnancy. The results for the last 6 days of pregnancy and the first 30 days postpartum are shown in Fig. 7.6. A similar pattern emerges as in the case of male no. C2 - there is no period of sexual inactivity immediately postpartum and copulatory behaviour also occurred at relatively high levels during late pregnancy. In this case, the female did show a postpartum ovulation, as evidenced by the hormone profiles shown in Fig. 7.6, though she did not conceive until the subsequent ovulation.

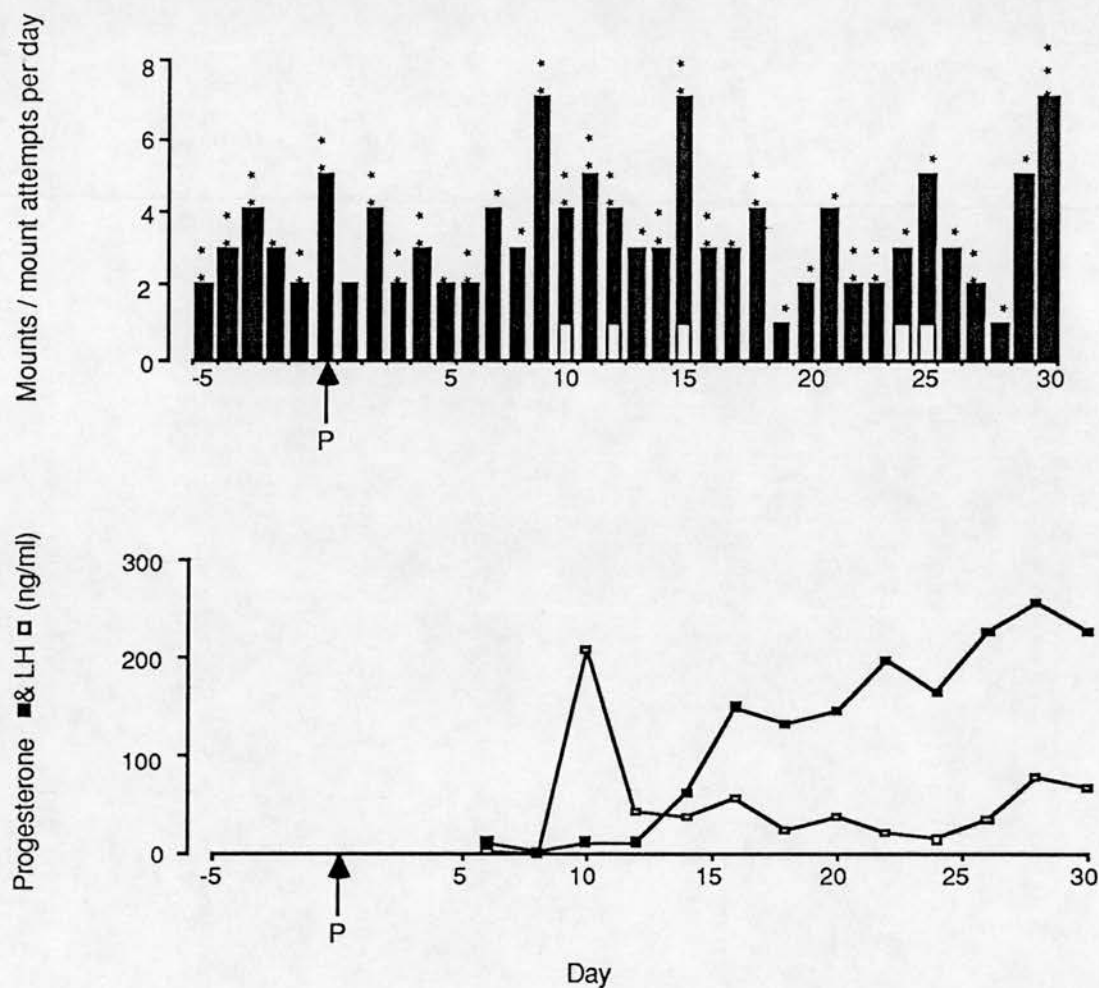


Fig. 7.6 Effects of an olfactory bulb section on the pre- and post-partum sexual behaviour between male no. C3 and his female partner in relation to the female's postpartum hormone profile.

The top histogram shows the number of mounts or attempted mounts during the 12 hour light period for each day. Male-initiated mounts = solid bars, female-initiated mounts = open bars. * = ejaculation. Plasma hormone levels are shown in ng/ml in the lower graph. P = day of parturition.

As shown in Figs. 7.5 and 7.6, female-initiated mounts occurred at low frequencies in both cases and there did not appear to be a correlation between levels of proceptive behaviour and postpartum ovulation for the female partner of male no. C3. Fig. 7.7 shows the distribution of mount refusals and terminations by the females across the study period. It appears that the maximum number of mount refusals and terminations by the females occurred during the immediate postpartum period (i.e. Days 1-10) when one would normally expect the sexual activity of the pairs to be minimal. It is interesting that the female partner of male no. C3 did not refuse any of the male's mount attempts during the 6 days preceding parturition despite the imminent birth of the infants.

Male no. C2

Day	No. Mounts / Mount attempts	% Refused / Terminated
0-10	16	25
11-20	31	9.7
21-30	25	4.2

Male no. C3

Day	No. Mounts / Mount attempts	% Refused / Terminated
-5-0	19	0
1-10	35	17.1
11-20	34	2.9
21-30	33	6.1

Fig. 7.7 Distribution of mount refusals and terminations by the female partners of male nos. C2 and C3 during the pre- and post-partum period.

7.3.6 Effects on general health

The males remained in perfect health following surgery and there were no

adverse effects on either eating or drinking behaviour. Indeed, the body weight of the two males remained within 5% of the pre-operative values throughout the post-operative period. Mann-Whitney U-tests within animals reveal significant declines in scent marking during the first hour of each day for male no. C2 (mean \pm s.e.m. from 8.08 \pm 0.91 to 2.97 \pm 0.47, $n=30$, $U=107.5$, $P<0.001$) and male no. C3 (from 4.37 \pm 0.49 to 2.77 \pm 0.50, $n=30$, $U=288$, $P<0.05$), though the general activity levels of the two males appeared completely normal during the observation periods.

7.4 Discussion

No generalised conclusions can be drawn from the data quoted in this chapter as only 2 males were studied. However, within these 2 animals, anosmia resulted in a number of interesting behavioural changes which indicate that this sensory system should be investigated in more detail in marmosets in the future.

Although male no. C3 showed a significant decline in infant carrying behaviour following sectioning of the olfactory bulbs, the effects following this surgery do not correspond to those seen following POA-AH lesions (see Chapter 6). In the present study, the males did not actively avoid the infants, as evidenced by the percentage of male carrying bouts terminated by dislodge behaviour, and by the number of infant-initiated transfers that the two males refused. These two indexes of male avoidance of the infants corresponded closely to the results obtained during the pre-operative postpartum period and, in this respect, these males contrast sharply with the males that received POA-AH lesions. Of course it is possible that the significant decline in the carrying behaviour of male no. C3 was the result of

the increase in carrying behaviour shown by his female partner and not *vice versa*. Indeed, it is known that the parental behaviour shown by individuals of this, and other marmoset species, varies with successive pregnancies (Epple, 1975b; Ingram, 1977; Vogt et al., 1978).

It therefore appears from this preliminary study that the olfactory system does not provide crucial sensory information for the expression of paternal behaviour in the common marmoset. This lack of an effect of anosmia on paternal care contrasts with results seen in other mammalian species in which both potentiation and suppression of maternal behaviour result from this procedure - see Chapter 2. However, much of the work carried out on the role of olfaction in parental behaviour has employed rats which are macrosmatic and therefore highly dependent on olfactory cues from their environment. In comparison, marmosets possess a more highly developed visual system which may have led to a reduced dependency on olfactory cues.

However, olfaction is obviously still of importance in this species, as evidenced by the levels of scent marking seen in captive groups, by the fact that marmosets can recognise a strange animal's sex and dominance status by its scent mark (Epple, 1970b;1975a; Sutcliffe & Poole, 1978), and by the effects of anosmia on other behavioural patterns in the present study. Grooming behaviour by the males directed towards the female partner and towards the infants was severely disrupted following olfactory bulb sections, as was the incidence of allogrooming by the females directed towards the males. These dramatic decreases in grooming behaviour are opposite to the effects of the POA-AH lesions described in Chapter 6. It has been argued that both allogrooming and anogenital grooming provide an important positive social interaction between members of a group of common marmosets that serves to strengthen bonds between individuals -

see Chapters 4 and 6. It is likely that olfactory cues play an important role in the initiation of grooming behaviour. The observed declines in female allogrooming may have been due to a decrease in the number of grooming invitations exhibited by the males but unfortunately the data needed to support this hypothesis were not collected. In earlier Chapters (4 and 6), it was argued that the increases in grooming behaviour seen following POA-AH lesions were a form of displacement activity caused by a decline in levels of sexual interactions between adult pairs, or by declines in affiliative behaviour in the form of carrying behaviour between males and infants. It is interesting that in the present study, in which sharp declines in grooming behaviour between members of the group were seen, sexual behaviour occurred throughout the pre- and immediate post-partum periods at higher levels than were expected. Grooming behaviour may require olfactory feedback and not just tactile feedback for its maintenance which would explain the decrease in male allogrooming and anogenital grooming seen following olfactory bulb sections, but would not explain the decrease in the amount of time that the females spent grooming their male partners.

Anosmia also results in a decline in the scent marking activity of the two males. Scent marking normally occurs maximally following introduction of animals into a novel environment, during aggressive and sexual encounters (Epple, 1970b;1975a) or in response to the scent marking of other individuals, i.e. animals are frequently seen to scent mark on top of a previous mark of another animal (personal observation). Therefore, it is presumably the lack of perception of novel odours and of the scent marks of other individuals which leads to a decline in the scent marking exhibited by anosmic males.

The effects of male anosmia on the postpartum sexual activity of these animals was also interesting. It has been postulated that an olfactory cue

produced by the female coincidently with the onset of postpartum ovarian activity is responsible for the rise in sexual activity during this period (Dixon & Lunn, 1987). However, the present study suggests a second hypothesis, as sexual behaviour continued throughout the 30 day postpartum period without any overt behavioural cue from the females and without the possibility of the males responding to olfactory cues. It has been noted that copulation continues throughout pregnancy in this species, and in other Callitrichids, and that there is a tendency to increased levels during pregnancy and immediately prior to parturition (Evans & Poole, 1984; Goldizen, 1986). However, parturition is normally followed by a period of sexual inactivity associated with a higher number of mounts refused or terminated by the females (Dixon & Lunn, 1987) and by a lower number of mount attempts by the males (see Chapter 6). In contrast, in this study, despite a higher percentage of mounts refused or terminated by the female during the first 10 days postpartum, sexual activity continued at a similar level to that seen during the later stages of the study i.e. days 11-30 postpartum or during the late prepartum period in the case of male no. C3. This clearly indicates that the anosmic males were initiating a larger number of mounts than normal during this period. It is therefore possible that an olfactory cue from the female is used to inhibit sexual activity during this period, and not to reinitiate copulation once the female has begun to cycle. It is possible that the hormonal changes coincident with parturition serve to render the female sexually unattractive to the male for a number of days and that this unattractiveness disappears with the resumption of ovarian activity. There is no evidence in the literature to suggest that females are actually unattractive to males immediately postpartum however so, at this stage, it is mere speculation. One way of investigating this hypothesis would be to carry out a series of pair tests on a daily basis between a normal male and

an ovariectomised female which had been rubbed with the scent of a second female who had just given birth. The scent could be changed each day to present the male with the same olfactory cues from the ovariectomised female as he would normally receive from an intact female during the postpartum period. If there was a decrease in sexual activity between the pair coincident with the scent cues from the intact female immediately postpartum, it would suggest that a negative olfactory cue exists at this time. However, in a pair test situation mating continues throughout the ovarian cycle (Kendrick & Dixon, 1983) though the validity of comparing sexual behaviour exhibited during pair tests with that shown between a permanent partnership is questionable. It is also of interest that relatively high levels of mating activity continued up to 30 days postpartum, despite the fact that neither of the females conceived at this time and this has been found to lead to reduced levels of sexual activity following ovulation in an earlier study (Dixon & Lunn, 1987) though not in the study described in Chapter 6. Of interest here is the observation that rhesus monkeys continue to show rhythms of sexual activity through the ovarian cycle after the olfactory epithelium of the males has been destroyed (Goldfoot et al., 1978). This report is unlike the findings here in which the postpartum patterning of sexual activity in marmosets is disrupted by the anosmia induced in the males.

Another interpretation of the data from normal pairs is that the lower level of sexual receptivity exhibited by these females during the immediate postpartum period serves to deter mounting attempts by the male. However, if this were the case, a similar decline would be expected during the present study in which the females also exhibited lower levels of receptivity during the first 10 days postpartum. It is therefore likely that the inactivity normally seen at this time is associated with an olfactory cue rather than with a

behavioural one.

In contrast to a former study (Dixon & Lunn, 1987), sexual behaviour continued between male no. C2 and his female partner throughout the 30 day postpartum period despite the fact that the female did not cycle during this time. However, it is well known that this species will readily copulate during pair tests even if the female has been ovariectomised and received no hormonal treatment, indicating that normal levels of ovarian hormones are not essential for the expression of sexual behaviour, at least in a pair test situation (Kendrick & Dixon, 1984b). It also seems, given the unusual conditions of the present study, that ovarian hormones are not essential for the expression of receptivity by females in permanent social groups.

Although female marmosets have been found to exhibit higher levels of proceptivity and receptivity around the time of ovulation (Kendrick & Dixon, 1983), no similar pattern was seen in the case of male no. C3's partner in which a postpartum ovarian cycle occurred. However, in this testing situation where the pairs were in constant contact, very little proceptivity (as evidenced by female-initiated mounts) was seen at any stage of the cycle. Thus, the effects of ovarian hormones on this behavioural pattern may be more evident in a pair test situation though, in the previous study (Dixon & Lunn, 1987), some females did show a marked peak in proceptivity coincident with the postpartum LH surge.

CHAPTER 8

GENERAL DISCUSSION

The conclusions drawn from each study have been discussed in detail in the relevant chapters, therefore the purpose of this chapter is to bring together conclusions and comments which arise from an overview of the data presented in this thesis.

This series of experiments was undertaken in an attempt to elucidate some aspects of the neural control of masculine reproductive and social behaviours in the common marmoset. The hypothalamus, and the POA-AH in particular, proved to be fascinating in this respect. It is therefore with an hypothesis of the precise role of the POA-AH in the control of a number of behaviours that this discussion begins.

From the results of sexual behaviour following POA-AH lesions, it is clear that sexual arousal has been affected in males in the pair test and the permanent group situation (see Chapters 4 and 6). Copulatory behaviour also dramatically declined following these lesions and this may have been a direct effect on copulatory behaviour *per se* or purely a result of the deficit in sexual arousal. Other aspects of the males' social behaviour directed towards their female partners did not decline following these lesions. It is interesting that this appears to be in conflict with the results from studies in other species in which aspects of masculine sexual arousal are spared following MPOA-AH lesions, for example, the 'flehmen' response in male goats (Hart, 1986) and ultrasonic precopulatory vocalisations in male mice (Bean et al., 1981). However, there are problems involved in quantifying sexual arousal and interest in animals and it is possible that some of the behaviours used as indicators of this phenomenon are incorrectly characterised. For example, Slimp et al. (1978) report that male rhesus monkeys continue to exhibit masturbatory behaviour in their home cages following MPOA-AH lesions. However, this behaviour occurred in visual isolation from the females and it is therefore difficult to reconcile this with the

normal response of a male to the sight, smell and tactile stimulus of a female partner. In retrospect, it would have been valuable to test the lesioned males in the present study in a more refined way to quantify their sexual interest in females. This may have been possible under conditions where the males were in olfactory and visual contact with their female partners but copulation was impossible. However, it is of interest that increasing the proceptivity of the females elicited no sexual response from these males.

Yoshimatsu's electrophysiological experiments with male rhesus macaques add an elegant new perspective to thoughts on the hypothalamic control of masculine sexual behaviour in primates; DMH and PH neurons fire more frequently during mounting and thrusting and he suggests that they contribute in some way to the 'copulatory mechanism' (Yoshimatsu, 1983). Perachio et al. (1979) had previously shown remarkable effects of stimulating the DMH in freely moving rhesus monkeys upon copulatory behaviour. In the present study, more medial lesions resulted in a continuation of mounting and thrusting but an apparent inability to intromit on the majority of post-operative tests. It would clearly be of interest to study the effects of medial and dorsal hypothalamic lesions in more detail in this species in the future.

A valuable opportunity to evaluate the precise role of the MPOA-AH in the control of masculine sexual behaviour in clinical studies of the human male has been wasted. Some studies have involved lesioning the hypothalamus in sex offenders (e.g. Meyers, 1961; 1962). Although lesions in the AH or medial hypothalamus were reported to reduce sexual activity, no quantitative data on sexual desire and copulatory behaviour were collected. However, the ethics of such approaches are questionable and these operations are rarely performed.

It is noticeable from the data quoted in this thesis that the most profound

effects upon sexual behaviour followed lesions situated beneath the anterior commissure at the junction of the POA and AH. In this area there is a large nucleus - the anterodorsal nucleus - which stains densely with cresyl violet. It is prominent in both sexes but there is no quantitative evidence as to whether its overall size, neuronal density or cell body size differs between males and females. It would clearly be of interest to examine this area and to evaluate the role of this nucleus more specifically in the control of masculine sexual behaviour. In a preliminary study, bilateral infusions of ibotenic acid (which specifically destroys cell bodies) into the POA-AH of male marmosets have produced similar effects to the thermal lesions reported in this thesis (A.F. Dixon & S.A.C. Lloyd, unpublished data).

In the preliminary study on agonistic behaviour, it appears that lesions within the POA-AH also result in a decrease in the aggressive behaviour directed towards an unfamiliar male intruder (see Chapter 4). Interestingly, it was the males that suffered the severest declines in sexual behaviour that also revealed the largest deficits in agonistic behaviour. Indeed, in some post-operative tests, the resident males virtually ignored the presence of the unknown male, or actually interacted positively with the intruder in the form of allogrooming.

The results from the study on the effects of POA-AH lesions upon paternal behaviour are equally conclusive (see Chapter 6). Male common marmosets do not carry their offspring following these lesions though they will interact with them in other ways such as allogrooming. In the discussion in Chapter 6, it was argued that carrying behaviour is the only purely paternal behaviour exhibited by these males, as all other aspects of the behaviour directed towards infants is also observed between all members of a group.

The uniting factor between these three sets of results is that it appears to be

the behaviours that are dependent on the sex and age of the stimulus animal that are specifically affected following lesions within the POA-AH. For example, under normal conditions, sexual behaviour will only be directed towards an adult female, aggressive behaviour is more likely to occur between unfamiliar males, and carrying behaviour i.e. paternal behaviour is only appropriate when directed towards infants. It is therefore possible that the POA-AH in some way acts as a relay between the input of sensory and emotional information regarding the nature of the stimulus animal and the output of the appropriate behavioural response.

The anatomical position of the POA-AH lends support to this hypothesis. As described in Chapter 1, the anterior hypothalamus has major inputs from areas of the cortex and olfactory system via the amygdalae and stria terminalis, and numerous efferent connections with other areas of the hypothalamus and more caudal neural structures via a number of fibre tracts, principally the medial forebrain bundle.

The role of the amygdalae in the control of social behaviour has been examined in a number of studies, as outlined in Chapter 2, and some interesting comparisons with the present study can be made. Early studies of amygdalectomies led to the view that this surgery results in hypersexuality in cats and monkeys (Schreiner & Kling, 1956; Kling, 1974). However, more recently the data have been re-examined and it is now argued that 'hypersexuality' is not the correct description. It would appear that animals are more prone to mount inappropriate objects rather than to express high levels of sexual activity *per se* and it is now thought that amygdalectomy results in 'inappropriate sexual behaviour' (Aronson & Cooper, 1979). These results indicate that the individuals are not recognising the stimulus object and thus not responding with the appropriate behaviour. From the work on the maternal behaviour of amygdalectomised monkeys described in

Chapter 2, it is again clear that these animals are not responding to infants with the appropriate behaviour, suggesting a lack of recognition.

The behaviour of animals following amygdectomy is more grossly abnormal than that of the males in the present study i.e. these lesioned males continue to behave competently as members of permanent social groups - in striking contrast to the effects of amygdectomy on rhesus monkeys (Kling, 1974). In this case, it appears that the lesioned males respond to all stimulus animals as a 'neuter', i.e. social interactions such as grooming continue at or above normal rates, though the behaviours that are dependent on the identity of the stimulus animal are abolished. It is therefore possible that these males recognise the presence of another marmoset, but are unable to relay the correct behavioural response.

The amygdalae and the POA-AH have major connections with the olfactory system and marmosets can gain much information about other individuals from olfactory cues (Epple, 1970b). It is therefore possible that these anatomical connections provide some of the sensory information that these males need in order to respond with the correct behaviour pattern. However, the results from Chapter 7 dispute this theory as these anosmic males continued to exhibit paternal behaviour towards the infants and sexual behaviour towards their female partners. It is notable, however, that the patterning of sexual activity is altered by olfactory bulb sections in male marmosets and their ability to concentrate ejaculatory activity around the periovulatory period is reduced by this operation. This contrasts with Goldfoot et al.'s study (1978) on rhesus monkeys in which ablation of the olfactory epithelium did not abolish the cyclicity of ejaculations during the female's menstrual cycle. An important point here is that primates are generally less dependent on olfactory cues than on visual cues. Indeed, the deficits seen in the maternal behaviour of more macrosmatic animals such

as rodents and ungulates during anosmia can be extensive - see Chapter 2. If the above theory is correct, one would expect to find that the amygdalae and POA-AH also have connections with the visual system and that these sensory inputs are of primary importance in primates. The traditional view is that visual inputs, processed by the cortex, must in some way impinge upon hypothalamic mechanisms which control sexual behaviour in primates (Herbert, 1974; 1981). More recently, electrophysiological single-unit recording studies have shown that neurons in the temporal cortex of monkeys (Perret et al., 1985) and sheep (Kendrick & Baldwin, 1987) fire specifically in response to the sight of faces. Certain 'face' cells are more responsive to particular features, e.g. the eyes or mouth, or to changes in gaze direction and facial expression. In addition, the inferotemporal cortex, where these cells are situated, is closely connected with the amygdalae and hippocampus and thence, via the stria terminalis, amygdalofugal pathway and fornix to the hypothalamus. It is therefore possible that these connections represent a way in which sensory inputs can influence the behavioural response elicited by the amygdalae and the POA-AH.

The presence of displacement activities has been discussed in Chapters 4 and 6, and this may lend support to the hypothesis that the POA-AH controls the appropriate behavioural response to a stimulus animal. It is clear that the lesioned males recognise other individuals as conspecifics because positive social interactions occur. One can argue that these individuals are therefore motivated to respond to the stimulus animal but are unable to distinguish the desired nature of that response. Therefore, allogrooming and grooming invitations, which are both appropriate positive social interactions between any individuals, emerge as a displacement activity for this thwarted motivation. It is interesting that female marmosets also exhibit higher levels of allogrooming and grooming invitations following hypothalamic lesions

which cause decreases in levels of proceptivity (Kendrick & Dixson, 1986). As discussed in Chapter 4, male rats also exhibit displacement activities following MPOA lesions (Hansen & Drake af Hagelsrum, 1984). The presence of displacement activities following MPOA lesions in female rats during maternal behaviour studies has not been reported in the literature.

There are a number of criticisms of the work described in this thesis. In particular, in some studies i.e. Chapters 6 and 7, the number of individuals used was small, despite the fact that 62 individuals were used during the course of this work (see Chapter 3). This was due, in part, to the length of time taken to study pairs of animals through the 30 day postpartum period, and also to the fact that there were a limited number of pairs in the breeding colony available for terminal studies. Another major problem arose regarding the stereotaxic placement of lesions and cannulae due to discrepancies between our animals and those used in the atlas. Despite a number of pilot studies and the use of a correction factor, the result of this discrepancy was a range of lesion and cannulae placements. This, in turn, resulted in the necessity of considering the males individually which limited the conclusions that could be inferred from the data. These points have been covered in more detail in the experimental chapters.

However, the results of POA-AH lesions and olfactory bulb sections were unequivocal enough to enable some conclusions to be drawn.

The hormonal data quoted in this thesis was limited and this could have led to inaccurate assumptions. However, it was decided that, during the parental behaviour studies at least, disturbance of the animals for frequent blood sampling could have led to detrimental behavioural effects. Also, plasma testosterone levels within each male are highly variable and this necessitates the collection of a large number of samples in order to produce meaningful results. Indirect evidence of the males' hormonal state was

gained from testicular histology and androgen-dependent morphology and this evidence supported the hypothesis inferred from the hormonal data that the behavioural changes seen following hypothalamic lesions were not the result of disruption of the hypothalamic-pituitary axis. These findings are in accord with work on MPOA-AH lesions on other mammals e.g. (Brookhart & Dey, 1941; Hart et al., 1973) and in the only other comparable study on primates, Slimp et al (1978) reported that plasma testosterone levels did not decline but give no quantitative data on this point.

As reviewed in Chapter 1, the hypothalamus controls a wide range of behavioural and homeostatic functions. It is therefore possible that the relatively gross lesioning techniques employed in these studies damaged a number of control systems. The evidence outlined in Chapters 4 and 6 suggests that the hypothalamic-pituitary-testicular axis is intact and functioning effectively but the pituitary gland releases a range of other hormones that have numerous effects throughout the body and it is possible that these control mechanisms have been disturbed. It seems unlikely that this is the case however, as the majority of the males that underwent these surgical techniques remained in perfect health even for relatively long post-operative periods, i.e. 6 months. One would assume that any gross hormonal abnormality would have become apparent during this time. The POA is closely involved in the control of temperature regulation - see Chapter 1, and in one case, male no. 9 in Chapter 4, experienced a significant fall in body temperature. This may have been directly due to an effect on the central control mechanisms or an indirect effect of an unrelated illness. The clearest non-behavioural effect of the lesions performed during this study were the increases in body weight experienced by almost all the animals. It is interesting that the males with the most medial lesions appeared to suffer the greatest increases. It is well known that the medial

hypothalamus is closely involved in the control of satiety, directly via the 'satiety centre' situated in the VmH, and indirectly via the release of insulin - see Chapter 1. However, the effects outlined above were minimal in all cases and it seems unlikely that they are causing the perceived behavioural changes following these hypothalamic lesions.

The hypothesis that the POA-AH influences the behavioural response to a given sensory and emotional input could be tested in a number of ways. It would be interesting to give males with POA-AH lesions choice tests between stimulus animals of different sexes and ages and to compare the choices made with those made by unoperated control males. This experiment would determine whether these lesioned males recognise that stimulus animals are different. Also, a series of tests could be designed in which lesioned males are presented with an unpredictable series of stimulus animals in a pair test situation and compare the speed and nature of their response to that of normal males.

Another line of investigation would be to study more of the neural pathways between the POA-AH and the cortex, via the stria terminalis and amygdalae, and the fornix and hippocampus. The effects of disconnecting these pathways by means of knife cuts would be of particular interest. One would expect the behavioural deficits to increase in magnitude as one proceeded towards the cortex. Anosmic males could also be used in an attempt to determine how much the olfactory system aids individual recognition in a primate which is generally considered to be more dependent on visual cues. It is also very important to compare the response of females to POA-AH lesions with that of males. It is known that POA-AH lesions lead to deficits in maternal behaviour - see Chapter 2, but it would be equally interesting to determine the wider social consequences of such surgery. Also, lesions in the female in a variety of hypothalamic sites cause decreases in proceptivity

(Kendrick & Dixson, 1986; Dixson & Lloyd, 1988).

A further line of investigation would be to determine which neurotransmitter systems are involved in this control mechanism. The POA-AH contains a wide range of neurotransmitters and is intricately connected with many intra- and extra-hypothalamic sites - see Chapters 1 and 2. It was hoped that the cannulation study described in Chapter 5 would give some insight into this aspect of the control system. Unfortunately, the results were not clear enough to support any firm conclusions regarding the role of oxytocin and β -endorphin within the POA-AH in the control of reproductive and social behaviours. These results indicate that the marmoset is a suitable primate for behavioural studies involving chronic cannulation and microinfusions into the hypothalamus. No previous studies have been carried out on primates to study the effects of intracerebral infusions of neuroactive peptides on behaviour in a freely moving subject. Although the results presented in this thesis are largely negative, it should be borne in mind that the approach is viable and provides an important avenue for future work. Thus, males have been shown to tolerate cannulae for periods of up to 7 months with little or no sign of infection, they can be trained to be held gently by hand during infusions only minutes before behavioural testing commences, and the cannulae are largely ignored by their female partners during pair tests. Clearly, more work is needed using more accurate cannula placements and a range of peptide dosages, but the studies reported here establish the feasibility of such projects.

REFERENCES

- ABBOTT, D.H. Differentiation of sexual behaviour in female marmoset monkeys: Effects of neonatal testosterone or a male co-twin.
Prog. Brain Res. (1984) 61 343-352.
- ABBOTT, D.H. & HEARN, J.P. Physical, hormonal and behavioural aspects of sexual development in the marmoset monkey, *Callithrix jacchus*.
J. Reprod. Fertil. (1978) 53 155-166.
- ABBOTT, D.H., HOLMAN, S.D., BERMAN, M., NEFF, D.A. & GOY, R.W. Effects of opiate antagonists on hormones and behavior of male and female rhesus monkeys.
Arch. Sex. Behav. (1984) 13 1-25.
- ABBOTT, D.H., MCNEILLY, A.S., LUNN, S.F., HULME, M.J. & BURDEN, F.J. Inhibition of ovarian function in subordinate female marmoset monkeys (*Callithrix jacchus jacchus*).
J. Reprod. Fertil. (1981) 63 335-345.
- ALBERTS, S. Parental care in captive Siamangs (*Hylobates syndactylus*).
Zoo Biology (1987) 6 401-406.
- ANAND, B.K. & BROBECK, J.R. Localization of a "feeding center" in the hypothalamus of the rat.
Proc. Soc. Exp. Biol. Med. (1951) 77 323-324.
- ARENDASH, G.W. & GORSKI, R.A. Testosterone-induced enhancement of male medial preoptic tissue transplant volume in female recipients: A possible neurotrophic action.
Dev. Brain Res. (1987) 34 69-77.
- ARGIOLAS, A., MELIS, M.R. & GESSA, G.L. Oxytocin: An extremely potent inducer of penile erection and yawning in rats.
Eur. J. Pharmacol. (1986) 130 265-271.
- ARONSON, L.R. & COOPER, M.L. Desensitization of the glans penis and sexual behavior in cats.
In: "Reproduction and Sexual Behavior." DIAMOND, M. (Ed.).
Indiana University Press (1968) 51-82.
- ARONSON, L.R. & COOPER, M.L. Olfactory deprivation and mating behavior in sexually experienced male cats.
Behav. Biol. (1974) 11 459-480.
- ARONSON, L.R. & COOPER, M.L. Amygdaloid hypersexuality in male cats re-examined.
Physiol. Behav. (1979) 22 257-265.
- AYOUB, D.M., GREENHOUGH, W.T. & JURASKA, J.M. Sex differences in dendritic structure in the preoptic area of the juvenile macaque monkey brain.
Science (1983) 219 197-198.

- BAILEY, D.J. & HERBERT, J. Impaired copulatory behaviour of male rats with hyperprolactinaemia induced by domperidone or pituitary grafts. *Neuroendocrinol.* (1982) 35 186-193.
- BARFIELD, R.J. Activation of sexual and aggressive behavior by androgen implanted into the male ring dove brain. *Endocrinol.* (1971) 89 1470-1476.
- BARFIELD, R.J. The hypothalamus and social behaviour with special reference to the hormonal control of sexual behaviour. *Poultry Sci.* (1979) 58 1625-1632.
- BAUM, M.J., KEVERNE, E.B., EVERITT, B.J. & HERBERT, J. Reduction of sexual interaction in rhesus monkeys by a vaginal action of progesterone. *Nature* (1976) 263 606-608.
- BEACH, F.A. The neural basis of innate behavior. I. Effects of cortical lesions upon the maternal behavior pattern in the rat. *J. Comp. Psychol.* (1937) 24 393-436.
- BEACH, F.A. Effects of cortical lesions upon the copulatory behavior of male rats. *J. Comp. Psychol.* (1940) 29 193-244.
- BEACH, F.A. Characteristics of the masculine 'sex drive'. In: "The Nebraska Symposium on Motivation. 4". JONES, M.R. (Ed.). Univ. Nebraska Press, Lincoln. (1956) 1-31.
- BEACH, F.A. Evolutionary aspects of psychoendocrinology. In: "Behavior and Evolution." ROE, A. & SIMPSON, G.G. (Eds.). Yale University Press. (1958) 81-102.
- BEAN, N.J., NUNEZ, A.A. & CONNER, R. Effects of medial preoptic lesions on male mouse ultrasonic vocalizations and copulatory behavior. *Brain Res. Bull.* (1981) 6 109-112.
- BEAN, N.J., NYBY, J., KERCHNER, M. & DAHINDEN, Z. Hormonal regulation of chemosignal-stimulated precopulatory behaviors in male housemice (*Mus musculus*). *Horm. Behav.* (1986) 20 390-404.
- BENKERT, O. Clinical studies on the effects of neurohormones on sexual behavior. In: "Sexual behavior: Pharmacology and biochemistry. SANDLER, M. & GESSA, G.L. (Eds.). Raven Press, New York. (1975) 297-305.
- BERENDSEN, H.H.G. & GOWER, A.J. Opiate-androgen interactions in drug-induced yawning and penile erections in the rat. *Neuroendocrinol.* (1986) 42 185-190.

- BERK, M.L. & FINKELSTEIN, J.A. Afferent projections to the preoptic area and hypothalamic regions in the rat brain. *Neurosci.* (1981) 6 1601-1624.
- BERNARD, C. Lecons sur les phenomenes de la vie communs aux animaux et aux vegetaux. Bailliere, Paris. (2 vols.) (1878/79).
- BERGQUIST, E.H. Output pathways of hypothalamic mechanisms for sexual, aggressive, and other motivational behaviors in the opossum. *J. Comp. physiol. Psychol.* (1970) 70 389-398.
- BERTOLINI, A., GESSA, G.L. & FERRARI, W. Penile erection and ejaculation: A central effect of ACTH-like peptides in mammals. In: 'Sexual Behavior: Pharmacology and Biochemistry.' SANDLER, M. & GESSA, G.L. (Eds.). Raven Press, New York. (1975) 247-257.
- BICKNELL, R.J. Endogenous opioid peptides and hypothalamic neuroendocrine neurones. *J. Endocrinol.* (1985) 107 437-446.
- BIELERT, C. Sexual interactions between captive adult male and female Chacma baboons (*Papio ursinus*) as related to the female's menstrual cycle. *J. Zool. Lond.* (1986) 209 521-536.
- BIELERT, C., HOWARD-TRIPP, M.E. & VAN DER WALT, L.A. Environmental and social factors influencing seminal emission in chacma baboons (*Papio ursinus*). *Psychoendocrinol.* (1980) 5 287-303.
- BITRAN, D. & HULL, E.M. Pharmacological analysis of male rat sexual behavior. *Neurosci. & Biobehav. Rev.* (1987) 11 365-389.
- BLUMER, D. Hypersexual episodes in temporal lobe epilepsy. *Amer. J. Psychiat.* (1970) 126 1099-1106.
- BOWERS, M.B., VAN WOERT, M. & DAVIS, L. Sexual behavior during L-dopa treatment for Parkinsonism. *Am. J. Psychiat.* (1971) 127 1691.
- BOX, H.O. Individual and intergroup differences in social behaviour among captive marmosets (*Callithrix jacchus*) and tamarins (*Saguinus mystax*). *Social Biology & Human Affairs* (1984) 47 49-68.
- BRACKETT, N.L. & EDWARDS, D.A. Medial preoptic connections with the midbrain tegmentum are essential for male sexual behavior. *Physiol. Behav.* (1984) 32 79-84.

- BREEDLOVE, S.M. & ARNOLD, A.P. Hormone accumulation in a sexually dimorphic motor nucleus in the rat spinal cord.
Science. (1980) 210 564-566.
- BREEDLOVE, S.M. & ARNOLD, A.P. Sexually dimorphic motor nucleus in the rat lumbar spinal cord: Response to adult hormone manipulation, absence in androgen-insensitive rats.
Brain Res. (1981) 225 297-307.
- BRONSON, F.H. & DESJARDINS, C. Endocrine responses to sexual arousal in male mice.
Endocrinol. (1982) 111 1286-1291.
- BROOKHART, J.M. & DEY, F.L. Reduction of sexual behavior in male guinea pigs by hypothalamic lesions.
Am. J. Physiol. (1941) 133 551-554.
- BROOKHART, J.M., DEY, F.L. & RANSON, S.W. The abolition of mating behavior by hypothalamic lesions in guinea pigs.
Endocrinol. (1941) 28 561-565.
- BUCHER, K., MYERS, R.E. & SOUTHWICK, C. Anterior temporal cortex and maternal behavior in monkey.
Neurology (1970) 20 415.
- BUIJS, R.M. Intra- and extra-hypothalamic vasopressin and oxytocin pathways in the rat. Pathways to the limbic system, medulla oblongata and spinal cord.
Cell Tiss. Res. (1978) 192 423-435.
- BYNE, W. & BLEIER, R. Medial preoptic sexual dimorphisms in the guinea pig. I. An investigation of their hormonal dependence.
J. Neurosci. (1987) 7 2688-2696.
- CAGGIULA, A.R. Analysis of the copulation-reward properties of posterior hypothalamic stimulation in male rats.
J. Comp. physiol. Psychol. (1970) 70 399-412.
- CAGGIULA, A.R., ANTELMAN, S.M. & ZIGMOND, M.J. Disruption of copulation in male rats after hypothalamic lesions: A behavioral, anatomical and neurochemical analysis.
Brain Res. (1973) 59 273-287.
- CAGGIULA, A.R., ANTELMAN, S.M. & ZIGMOND, M.J. Ineffectiveness of sexually arousing stimulation after hypothalamic lesions in the rat.
Physiol. Behav. (1974) 12 313-316.
- CARMICHAEL, M.S., HUMBERT, R., DIXEN, J. PALMISANO, G., GREENLEAF, W. & DAVIDSON, J.M. Plasma oxytocin increases in the human sexual response.
J. Clin. Endocr. Met. (1987) 64 27-31.

CHARLTON, M.H., JONES, A.J., WARD, B.J., DETTA A. & CLAYTON, R.N. Effects of castration and testosterone implants upon pituitary function in hypogonadal mice bearing normal foetal preoptic area grafts. *Neuroendocrinol.* (1987) 45 376-380.

CHEN, J.J. & BLISS, D.K. Effects of sequential preoptic and mammillary lesions on male rat sexual behavior. *J. Comp. physiol. Psychol.* (1974) 87 841-847.

CHRISTENSEN, L.W. & CLEMENS, L.G. Possible involvement of cyclic AMP in regulation of masculine sexual behaviour by testosterone. *J. Endocrinol.* (1974) 61 153-161.

CHRISTENSEN, L.W. & CLEMENS, L.G. Blockade of testosterone-induced mounting behavior in the male rat with intracranial application of the aromatization inhibitor, androst-1,4,6,-triene-3,17-dione. *Endocrinol.* (1975) 97 1545-1551.

CLARK, J.T., KALRA, S.P. & KALRA, P.S. Effects of a selective α_1 -adrenoceptor agonist, methoxamine, on sexual behavior and penile reflexes. *Physiol. Behav.* (1987) 40 747-753.

CLARK, J.T., SMITH, E.R. & DAVIDSON, J.M. Enhancement of sexual motivation in male rats by yohimbine. *Science* (1984) 225 847-849.

CLARK, J.T., SMITH, E.R. & DAVIDSON, J.M. Evidence for the modulation of sexual behavior by α -adrenoceptors in male rats. *Neuroendocrinol.* (1985) 41 36-43.

COMARR, A.E. & GUNDERSEN, B.B. Sexual function in traumatic paraplegia and quadriplegia. *Am. J. Nursing* (1975) 75 250.

COMMINS, D. & YAHR, P. Lesions of the sexually dimorphic area disrupt mating and marking in male gerbils. *Brain Res. Bull.* (1984) 13 185-193.

CONRAD, L.C.A. & PFAFF, D.W. Axonal projections of medial preoptic and anterior hypothalamic neurons. *Science* (1975) 190 1112-1114.

CURTIS, R.F., BALLANTINE, J.A., KEVERNE, E.B., BONSALE, R.W. & MICHAEL, R.P. Identification of primate sexual pheromones and properties of synthetic attractants. *Nature* (1971) 232 396-398.

CUSHMAN, P. Sexual behavior in heroin addiction and methadone maintenance. Correlation with plasma luteinizing hormone. *N.Y. State J. Medicine* (1972) 72 1261-1265.

- DAVIDSON, J.M. Activation of the male rat's sexual behavior by intracerebral implantation of androgen.
Endocrinol. (1966) 79 783-794.
- DAVIS, G.A., GOY, R.W. & POMERANTZ, S. Facilitation of penile erection in rhesus monkeys (*Macaca mulatta*) by the dopamine (D2) agonist, LY163502.
Am. J. Primatol. (1987) 12 338 (Abstract only).
- DAY, T.A., BLESSING, W. & WILLOUGHBY, J.O. Noradrenergic and dopaminergic projections to the medial preoptic area of the rat. A combined horseradish peroxidase / catecholamine fluorescence study.
Brain Res. (1980) 193 543-548.
- DE BRUIN, J.P.C., VAN OYEN, H.G.M., & VAN DE POLL, N. Behavioural changes following lesions of the orbital prefrontal cortex in male rats.
Behav. Brain Res. (1983) 10 209-232.
- DE JONGE, F.H., MEYERSON, B.J. & VAN DE POLL, N.E. Attractivity of male and female rats which are hormonally manipulated during early development and in adulthood.
Horm. Behav. (1986) 20 379-389.
- DE OLMOS, J.S. & INGRAM, W.R. The projection field of the stria terminalis in the rat brain. An experimental study.
J. Comp. Neurol. (1974) 146 303-334.
- DEVICHE, P. & MOORE, F.L. Steroidal control of sexual behavior in the rough-skinned newt (*Taricha granulosa*): Effects of testosterone, estradiol, and dihydrotestosterone.
Horm. Behav. (1988) 22 26-34.
- DIXSON, A.F. The hormonal control of sexual behaviour in primates.
Oxford Reviews of Reproductive Biology (1983a) 5 131-219.
- DIXSON, A.F. The owl monkey (*Aotus trivirgatus*).
In: "Reproduction in New World primates. HEARN, J.P. (Ed.).
MTP Press, Lancaster, Boston & The Hague. (1983b) 69-113.
- DIXSON, A.F. Plasma testosterone concentrations during postnatal development in the male common marmoset.
Folia Primatol. (1986) 47 166-170.
- DIXSON, A.F. Genital sensory feedback and sexual behaviour in male and female marmosets (*Callithrix jacchus*).
Physiol. Behav. (1986) 37 447-450.
- DIXSON, A.F. Observations on the evolution of the genitalia and copulatory behaviour in male primates.
J. Zool. Lond. (1987) 213 423-443.

- DIXSON, A.F. Effects of dorsal penile nerve transection upon the sexual behaviour of the male marmoset (*Callithrix jacchus*).
Physiol. Behav. (1988) 43 235-238.
- DIXSON, A.F. & FLEMING, D. Parental behaviour and infant development in Owl monkeys (*Aotus trivirgatus griseimembra*).
J. Zool., Lond. (1981) 194 25-39.
- DIXSON, A.F. & GEORGE, L. Prolactin and parental behaviour in a male New World primate.
Nature (1982) 299 511-553.
- DIXSON, A.F. & LLOYD, S.A.C. The hormonal and hypothalamic control of primate sexual behaviour.
Symp. Zool. Soc. Lond. (1988) 60 81-117.
- DIXSON, A.F. & LUNN, S.F. Post-partum changes in hormones and sexual behaviour in captive groups of marmosets (*Callithrix jacchus*).
Physiol. Behav. (1987) 41 577-583.
- DIXSON, A.F., SCRUTON, D.M. & HERBERT, J. Behaviour of the talapoin monkey (*Miopithecus talapoin*) studied in groups in the laboratory.
J. Zool. Lond. (1975) 176 177-210.
- DORSA, D.M., SMITH E.R. & DAVIDSON, J.M. Immunoreactive- β -endorphin and LHRH levels of aged male rats with impaired sex behavior.
Neurobiol. of Aging (1984) 5 115-120.
- EMERY, D.E. & SACHS, B.D. Copulatory behavior in male rats with lesions in the bed nucleus of the stria terminalis.
Physiol. Behav. (1976) 17 803-806.
- EPPLE, G. Maintenance, breeding, and development of marmoset monkeys (*Callithricidae*) in captivity.
Folia Primatol. (1970a) 12 56-76.
- EPPLE, G. Quantitative studies on scent marking in the marmoset (*Callithrix jacchus*).
Folia Primatol. (1970b) 13 48-62.
- EPPLE, G. The behavior of the marmoset monkey.
In: "Primate Behaviour. Vol. 4". ROSENBLUM, L.A. (Ed).
Academic Press, New York (1975a) 195-239.
- EPPLE, G. Parental behavior in *Saguinus fuscicollis* ssp. (*Callithricidae*).
Folia Primatol. (1975b) 24 221-238.
- EPPLE, G., ALVEARIO, M.C. & ST ANDRE E. Sexual and social behaviour of adult saddle-back tamarins (*Saguinus fuscicollis*), castrated as neonates.
Am. J. Primatol. (1987) 13 37-50.

- EVANS, I.M. & DISTILLER, L.A. Effects of luteinizing hormone-releasing hormone on sexual arousal in normal men.
Arch. Sex. Behav. (1979) 8 385-395.
- EVANS, S. & POOLE, T.B. Long-term changes and maintenance of the pair-bond in common marmosets, *Callithrix jacchus jacchus*.
Folia Primatol. (1984) 42 33-41.
- EVERITT, B.J. & STACEY, P. Studies of instrumental behavior with sexual reinforcement in male rats (*Rattus norvegicus*): II. Effects of preoptic area lesions, castration, and testosterone.
J. Comp. Psychol. (1987) 101 407-419.
- FABRE-NYS, C., MELLER, R.E. & KEVERNE, E.B. Opiate antagonists stimulate affiliative behaviour in monkeys.
Pharmacol. Biochem. & Behav. (1982) 16 653-659.
- FAHRBACH, S.E. & PFAFF, D.W. Effect of preoptic region implants of dilute estradiol on the maternal behavior of ovariectomised, nulliparous rats.
Horm. Behav. (1986) 20 354-363.
- FISHER, A.E. Maternal and sexual behavior induced by intracranial chemical stimulation.
Science (1956) 124 228-229.
- FJELLSTROM, D., KIHLLSTROM, J.E. & MELIN, P. The effect of synthetic oxytocin upon seminal characteristics and sexual behaviour in male rabbits.
J. Reprod. Fert. (1968) 17 207-209.
- FLEMING, A.S. & ROSENBLATT, J.S. Olfactory regulation of maternal behavior in rats: I Effects of olfactory bulb removal in experienced and inexperienced lactating and cycling females.
J. Comp. physiol. Psychol. (1974) 86 221-232.
- FLEMING, A.S., VACCARINO, F. & LUEBKE, C. Amygdaloid inhibition of maternal behavior in the nulliparous female rat.
Physiol. Behav. (1980) 25 731-743.
- FLEMING, A., VACCARINO, F., TAMBOSSO, L. & CHEE, P. Vomeronasal and olfactory system modulation of maternal behavior in the rat.
Science (1979) 203 372-374.
- FLETCHER, I.C. & LINDSAY, D.R. Sensory involvement in the mating behavior of domestic sheep.
Animal Behaviour (1968) 16 410-414.
- FLOODY, O.R. & PETROPOULOS, A.C. Aromatase inhibition depresses ultrasound production and copulation in male hamsters.
Horm. Behav. (1987) 21 100-104.

- FOX, C.A. & KNAGGS, G.S. Milk-ejection activity (oxytocin) in peripheral venous blood in man during lactation and in association with coitus. *J. Endocrinol.* (1969) 45 145-146.
- FRASER, H.M., LAIRD, N.C. & BLAKELEY, D.M. Decreased pituitary responsiveness and inhibition of the luteinizing hormone surge and ovulation in the stumptailed monkey (*Macaca arctoides*) by chronic treatment with an agonist of luteinizing hormone-releasing hormone. *Endocrinol.* (1980) 106 452-457.
- FUCHS, E., GLIESSMAN, P., HESS, D.L., PAU, K-Y.F., SPIES, H.G. & WUTTKE, W. Release rates of catecholamines, GABA and β -endorphin in the preoptic area and mediobasal hypothalamus of the rhesus monkey in push-pull perfusates: Correlation with blood hormone levels. *Exp. Brain Res.* (1986) 65 224-228.
- GANDEL MAN, R., ZARROW, M.X., DENENBERG, V.H. & MYERS, M. Olfactory bulb removal eliminates maternal behavior in the mouse. *Science* (1971) 171 210-211.
- GARCIA-VILLAR, R., SCHAMS, D., ALVINERIE, M., LAURENTIE, M.P. & TOUTAIN, P.L. Activity of the genital tract and plasma levels of oxytocin and cortisol at the time of mating in the ewe. *J. Endocrinol.* (1985) 105 323-329.
- GARDNER, J.E. & FISHER, A.E. Induction of mating in male chicks following preoptic implantation of androgen. *Physiol. Behav.* (1968) 3 709-712.
- GESSA, G.L., PAGLIETTI, E. & PELLEGRINI QUARANTOTTI, B. Induction of copulatory behavior in sexually inactive rats by naloxone. *Science* (1979) 204 203-205.
- GIANTONIO, G.W., LUND, N.L. & GERALL, A.A. Effect of diencephalic and rhinencephalic lesions on the male rat's sexual behavior. *J. Comp. physiol. Psychol.* (1970) 73 38-46.
- GINTON, A. & MERARI, A. Long range effects of MPOA lesions on mating behavior in the male rat. *Brain Res.* (1977) 120 158-163.
- GLICK, B.B., BAUGHMAN, W.L., JENSEN, J.N. & PHOENIX, C.H. Endogenous opiate systems and primate reproduction: Inability of naloxone to induce sexual activity in rhesus males. *Arch. Sex. Behav.* (1982) 11 267-275.
- GOLDFOOT, D.A., ESSOCK-VITALE, S.M., ASA, C.S., THORNTON, J.E. & LESHNER, A.I. Anosmia in male rhesus monkeys does not alter copulatory activity with cycling females. *Science* (1978) 199 1095-1096.

GOLDFOOT, D.A., KRAVETZ, M.A., GOY, R.W. & FREEMAN, S.K. Lack of effect of vaginal lavages and aliphatic acids on ejaculatory responses in rhesus monkeys: behavioral and chemical responses. *Horm. Behav.* (1976) 7 1-27.

GOLDIZEN, A.W. Tamarins and marmosets: Communal care of offspring. In: "Primate Societies." SMUTS, B.B., CHENEY, D.L., SEYFARTH, R.M., WRANGHAM, R.W. & STRUHSAKER, T.T. (Eds.). University of Chicago Press (1986) 34-43.

GORSKI, R.A., GORDON, J.H., SHRYNE, J.E. & SOUTHAM, A.M. Evidence for a morphological sex difference within the medial preoptic area of the rat brain. *Brain Res.* (1978) 148 333-346.

GOY, R.W. Role of androgens in the establishment and regulation of sex differences in mammals. *J. Anim. Sci.* (1966) 25 21-35.

GRAY, G.D., SMITH, E.R. & DAVIDSON, J.M. Hormonal regulation of penile erection in castrated male rats. *Physiol. Behav.* (1980) 24 463-468.

GREENHOUGH, W.T., CARTER, C.S., STEERMAN, C. & DE VOOGEL, T.J. Sex differences in dendritic patterns in male hamster preoptic area. *Brain Res.* (1977) 126 63-72.

GROSSMAN, R., DIEZ-GUERRA, F.J., MANSFIELD, S. & DYER, R.G. Neonatal testosterone modifies LH secretion in the adult female rat by altering the opioid-noradrenergic interaction in the medial preoptic area. *Brain Res.* (1987) 415 205-210.

GUERRA, M., RODRIGUEZ DEL CASTILLO, A., BATTANER, E. & MAS, M. Androgens stimulate preoptic area Na^+ , K^+ - ATPase activity in male rats. *Neurosci. Lett.* (1987) 78 97-100.

GUYTON, A.C. Textbook of Medical Physiology. W.B. Saunders Company, Philadelphia. (1981)

HAHN, E.F. & FISHMAN, J. Castration affects male rat brain opiate receptor content. *Neuroendocrinol.* (1985) 41 60-63.

HANSEN, S. Hypothalamic control of motivation: The medial preoptic area and masculine sexual behaviour. *Scand. J. Psychol.* (1982a) 1 121-126.

HANSEN, S. Spinal control of sexual behavior: Effects of intrathecal administration of lisuride. *Neurosci. Lett.* (1982b) 33 329-332.

HANSEN, S. & DRAKE AF HAGELSRUM, J.L.K. Emergence of displacement activities in the male rat following thwarting of sexual behavior. *Behav. Neurosci.* (1984) 98 868-883.

HANSEN, S. & KOHLER, C. The importance of the peripeduncular nucleus in the neuroendocrine control of sexual behavior and milk ejection in the rat. *Neuroendocrinol.* (1984) 39 563-572.

HANSEN, S., KOHLER, CH., GOLDSTEIN, M. & STEINBUSCH, H.V.M. Effects of ibotenic acid-induced neuronal degeneration in the medial preoptic area and the lateral hypothalamic area on sexual behavior in the male rat. *Brain Res.* (1982a) 239 213-232.

HANSEN, S., KOHLER, C. & ROSS, S. On the role of the mesencephalic tegmentum in the control of masculine sexual behaviour in the rat: Effects of electrolytic lesions, ibotenic acid and DSP4. *Brain Res.* (1982b) 240 311-320.

HARRIS, V.S. & SACHS, B.D. Copulatory behavior in male rats following amygdaloid lesions. *Brain Res.* (1975) 86 514-518.

HART, B.L. Sexual reflexes and mating behavior in the male dog. *J. Comp. physiol. Psychol.* (1967) 64 388-399.

HART, B.L. Alteration of quantitative aspects of sexual reflexes in spinal male dogs by testosterone. *J. Comp. physiol. Psychol.* (1968) 66 726-730.

HART, B.L. Effects of testosterone propionate and dihydrotestosterone on penile morphology and sexual reflexes of spinal male rats. *Horm. Behav.* (1973) 4 239-246.

HART, B.L. Medial preoptic-anterior hypothalamic area and sociosexual behavior of male dogs: A comparative neuropsychological analysis. *J. Comp. physiol. Psychol.* (1974) 86 328-349.

HART, B.L. Sequential medial preoptic-anterior hypothalamic lesions have same effect on copulatory behavior of male cats as simultaneous lesions. *Brain Res.* (1980) 185 423-428.

HART, B.L. Medial preoptic-anterior hypothalamic lesions and sociosexual behavior of male goats. *Physiol. Behav.* (1986) 36 301-305.

HART, B.L. & HAUGEN, C.M. Scent marking and sexual behavior maintained in anosmic male dogs. *Comm. Behav. Biol.* (1971) 6 131-135.

HART, B.L., HAUGEN, C.M. & PETERSON, D.M. Effects of medial preoptic-anterior hypothalamic lesions on mating behavior of male cats. *Brain Res.* (1973) 54 177-191.

HART, B.L. & LADEWIG, J. Effects of medial preoptic-anterior hypothalamic lesions on development of sociosexual behavior in dogs. *J. Comp. physiol. Psychol.* (1979) 93 566-573.

HART, B.L. & LEEDY, M.G. Neurological basis of male sexual behavior: A comparative analysis. In: "Handbook of Behavioral Neurobiology. 7. Reproduction." ADLER, N., PFAFF, D. & GOY, R.W. (Eds.). Plenum Press, New York. (1985) 373-422.

HASHIMOTO, R. & KIMURA, F. Inhibition of gonadotropin secretion induced by cholecystinin implants in the medial preoptic area by the dopamine receptor blocker, pimozide, in the rat. *Neuroendocrinol.* (1986) 42 32-37.

HASTINGS, M.H., WINN, P. & DUNNETT, S.B. Neurotoxic amino acid lesions of the lateral hypothalamus: A parametric comparison of the effects of ibotenate, N-methyl-D,L-aspartate and quisqualate in the rat. *Brain Res.* (1985) 360 248-256

HAUSFATER, G. & TAKACS, D. Structure and function of hindquarter presentations in yellow baboons (*Papio cynocephalus*). *Ethology* (1987) 74 297-319.

HAYWARD, J.N. Functional and morphological aspects of hypothalamic neurons. *Physiol. Rev.* (1977) 57 574-658.

HEIMER, L. & LARSSON, K. Impairment of mating behavior in male rats following lesions in the pre-optic - anterior hypothalamic continuum. *Brain Res.* (1966/67) 3 248-263.

HEIMER, L. & NAUTA, W.J.H. The hypothalamic distribution of the stria terminalis in the rat. *Brain Res.* (1969) 13 284-297.

HENDRICKS, S.E & SCHEETZ, H.A. Interaction of hypothalamic structures in the mediation of male sexual behavior. *Physiol. Behav.* (1973) 10 711-716.

HERBERG, L.J. Seminal ejaculation following positively reinforcing electrical stimulation of the rat hypothalamus. *J. Comp. physiol. Psychol.* (1963) 56 679-685.

HERBERT, J. Some functions of hormones and the hypothalamus in the sexual activity of primates. *Prog. Brain Res.* (1974) 41 331-348.

HERBERT, J. The role of the dorsal nerves of the penis in the sexual behaviour of the male rhesus monkey.
Physiol. Behav. (1973) 10 293-300.

HERBERT, J. Hormones and sexual strategies of primates.
Symp. Zool. Soc. Lond. (1981) 46 337-359.

HERSHKOVITZ, P. Living New World monkeys (Platyrrhini) - Vol. 1
University of Chicago Press. (1977).

HESS, J.P. Some observations on the sexual behaviour of captive lowland gorillas. *Gorilla g. gorilla*.
In: 'Comparative Ecology and Behaviour of Primates'. MICHAEL, R.P. & CROOK, J.H. (Eds.).
Academic Press, New York. (1973) 508-581.

HESS, W.R. Diencephalon: Autonomic and Extrapyrmidal Functions.
Grune & Stratton, New York. (1954).

HETHERINGTON, A.W. & RANSON, S.W. The spontaneous activity and food intake of rats with hypothalamic lesions.
Am. J. Physiol. (1942) 136 609-617.

HILLARP, N-A., OLIVECRONA, H. & SILFVERSKIOLD, W. Evidence for the participation of the preoptic area in male mating behaviour.
Experientia (1954) 5 224-225.

HINES, M., DAVIS, F.C., COQUELIN, A., GOY, R.W. & GORSKI, R.A. Sexually dimorphic regions in the medial preoptic area and the bed nucleus of the stria terminalis of the guinea pig brain. A description and an investigation of their relationship to gonadal steroids in adulthood.
J. Neurosci. (1985) 5 40-47.

HITT, J.C., BRYON, D.M. & MODIANOS, D.T. Effects of rostral medial forebrain bundle and olfactory tubercle lesions upon sexual behavior of male rats.
J. Comp. physiol. Psychol. (1973) 82 30-36.

HITT, J.C., HENDRICKS, S.E., GINSBERG, S.I. & LEWIS, J.H. Disruption of male, but not female, sexual behavior in rats by medial forebrain bundle lesions.
J. Comp. physiol. Psychol. (1970) 73 377-384.

HO, G.K-W., QUADAGNO, D. & MOLTZ, H. Intracranial cycloheximide: Effects on maternal behavior in the postparturient rat.
Pharmacol. Biochem. Behav. (1974) 2 455-458.

HORI, T., KIYOHARA, T., OOMURA, Y., NISHINO, H., AOU, S. & FUJITA, I. Activity of thermosensitive neurons of monkey preoptic hypothalamus during thermoregulatory operant behavior.
Brain Res. Bull. (1987) 18 649-655.

HUBRECHT, R.C. Field observations on group size and composition of the common marmoset (*Callithrix jacchus jacchus*), at Tapacura, Brasil. *Primates* (1984) 25 13-21.

HUGHES, A.M., EVERITT, B.J. & HERBERT, J. Selective effects of β -endorphin infused into the hypothalamus, preoptic area and bed nucleus of the stria terminalis on the sexual and ingestive behaviour of male rats. *Neurosci.* (1987) 23 1063-1073.

HUTCHISON, J.B. Effects of hypothalamic implants of gonadal steroids on courtship behaviour in barbary doves (*Streptopelia risoria*). *J. Endocrinol.* (1971) 50 97-113.

INGRAM, J.C. Interactions between parents and infants, and the development of independence in the common marmoset (*Callithrix jacchus*). *Anim. Behav.* (1977) 25 811-827.

JACOBSEN, C.D., ARNOLD, A.P. & GORSKI, R.A. Steroid autoradiography of the sexually dimorphic nucleus of the preoptic area. *Brain Res.* (1987) 414 349-356.

JACOBSEN, C.D. & GORSKI, R.A. Neurogenesis of the sexually dimorphic nucleus of the preoptic area in the rat. *J. Comp. Neurol.* (1981) 196 519-529.

JACOBSEN, C.D., TERKEL, J., GORSKI, R.A. & SAWYER, C.H. Effects of small medial preoptic area lesions on maternal behavior: Retrieving and nest building in the rat. *Brain Res.* (1980) 194 471-478.

JARZAB, B., SICKMOLLER, P.M., GEERLINGS, H. & DOHLERS, K.D. Postnatal treatment of rats with adrenergic receptor agonists or antagonists influences differentiation of sexual behaviour. *Horm. Behav.* (1987) 21 478-492.

JOHNSTON, P. & DAVIDSON, J.M. Intracerebral androgens and sexual behavior in the male rat. *Horm. Behav.* (1972) 3 345-357.

JONES, J. *The Mysteries of Opium Revealed.* Oxford University Press. (1700).

KALRA, P.S., SIMPKINS, J.W., LUTTGE, W.G. & KALRA, S.P. Effects on male sex behavior and preoptic dopamine neurons of hyperprolactinaemia induced by MtTW15 pituitary tumors. *Endocrinol.* (1983) 113 2065-2071.

KAMEL, F., MOCK, E.J., WRIGHT, W.W. & FRANKEL, A.I. Alterations in plasma concentrations of testosterone, LH, and prolactin associated with mating in the male rat. *Horm. Behav.* (1975) 6 277-288.

KANDEL, E.R. & SCHWARTZ, J.H. Principles of neural science. Elsevier, New York (1985).

KEESEY, R.E., BOYLE, P.C., KEMNITZ, J.W. & MITCHEL, J.S. The role of the lateral hypothalamus in determining the body weight set point. In: "Hunger: Basic Mechanisms and Clinical Implications." NOVIN, D., WYRWICKA, W. & BRAY, G.A. (Eds.). Raven Press, New York. (1976) 243-255.

KENDRICK, K.M. & BALDWIN, B.A. Cells in temporal cortex of conscious sheep can respond preferentially to the sight of faces. Science (1987) 236 448-450.

KENDRICK, K.M. & DIXSON, A.F. The effect of the ovarian cycle on the sexual behaviour of the common marmoset. Physiol. Behav. (1983) 30 735-742.

KENDRICK, K.M. & DIXSON, A.F. A quantitative description of copulatory and associated behaviors of captive marmosets (*Callithrix jacchus*). Int. J. Primatol. (1984a) 5 199-212.

KENDRICK, K.M. & DIXSON, A.F. Ovariectomy does not abolish proceptive behaviour cyclicity in the common marmoset. J. Endocrinol. (1984b) 101 155-162.

KENDRICK, K.M. & DIXSON, A.F. Hyperprolactinaemia abolishes sensitivity of stria terminalis neurones to testosterone. J. Endocrinol. (1984c) 102 109-113.

KENDRICK, K.M. & DIXSON, A.F. Effects of oestradiol 17 β , progesterone and testosterone upon proceptivity and receptivity in ovariectomised common marmosets. Physiol. Behav. (1985a) 34 123-128.

KENDRICK, K.M. & DIXSON, A.F. LHRH enhances proceptivity in a primate. Neuroendocrinol. (1985b) 41 449-453.

KENDRICK, K.M. & DIXSON, A.F. Anteromedial hypothalamic lesions block proceptivity but not receptivity in the female common marmoset. Brain Res. (1986) 375 211-229.

KENDRICK, K.M., KEVERNE, E.B., CHAPMAN, C. & BALDWIN, B.A. Intracranial dialysis measurement of oxytocin, monoamine and uric acid release from the olfactory bulb and substantia nigra of sheep during parturition, suckling, separation from lambs and eating. Brain Res. (1988a) 439 1-10.

- KENDRICK, K.M., KEVERNE, E.B., CHAPMAN, C. & BALDWIN, B.A. Microdialysis measurement of oxytocin, aspartate, γ -aminobutyric acid and glutamate release from the olfactory bulb of the sheep during vaginocervical stimulation. *Brain Res.* (1988b) 442 171-174.
- KENDRICK, K.M., KEVERNE, E.B. & BALDWIN, B.A. Intracerebroventricular oxytocin stimulates maternal behaviour in the sheep. *Neuroendocrinol.* (1987) 46 56-61.
- KENDRICK, K.M., KEVERNE, E.B., BALDWIN, B.A. & SHARMAN, D.F. Cerebrospinal fluid levels of acetylcholine, monoamines and oxytocin during labour, parturition, vaginocervical stimulation, lamb separation and suckling in sheep. *Neuroendocrinol.* (1986) 44 149-156.
- KIMBLE, D.P., ROGERS, L. & HENDRICKSON, C.W. Hippocampal lesions disrupt maternal, not sexual, behavior in the albino rat. *J. Comp. physiol. Psychol.* (1967) 63 401-407.
- KINSLEY, C.H. & BRIDGES, R.S. Prenatal stress and maternal behavior in intact virgin rats: Response latencies are decreased in males and increased in females. *Horm. Behav.* (1988a) 22 76-89.
- KINSLEY, C.H. & BRIDGES, R.S. Prolactin modulation of the maternal-like behavior displayed by juvenile rats. *Horm. Behav.* (1988b) 22 49-65.
- KLARIC, J.S. & HENDRICKS, S.E. Effects of two-stage lesions of the medial preoptic area on sexual behavior in male rats. *Physiol. Behav.* (1986) 37 539-542.
- KLING, A. Differential effects of amygdectomy in male and female nonhuman primates. *Arch. Sex. Behav.* (1974) 3 129-134.
- KLUVER, H. & BUCY, P.C. "Psychic blindness" and other symptoms following bilateral temporal lobectomy in Rhesus monkeys. *Am. J. Physiol.* (1937) 119 352-353.
- LAMB, M.E. Physiological mechanisms in the control of maternal behavior in rats: A review. *Psychol. Bull.* (1975) 82 104-119.
- LANG, R.E., HEIL, J., GANTEN, D., HERMANN, K., RASLHER, W. & UNGER, TH. Effects of lesions in the paraventricular nucleus of the hypothalamus on vasopressin and oxytocin contents in brainstem and spinal cord of rat. *Brain Res.* (1983) 260 326-329.

- LARSSON, K. Features of the neuroendocrine regulation of masculine sexual behaviour.
In: "Comprehensive Endocrinology. Endocrine Control of Sexual Behaviour. BEYER (ed).
Raven Press (1979) 77-166.
- LARSSON, K., SODERSTEN, P. & BEYER, C. Sexual behavior in male rats treated with estrogen in combination with dihydrotestosterone.
Horm. Behav. (1973) 4 289-299.
- LEEDY, M.G., VELA, E.A., POPOLOW, H.B. & GERALL, A.A. Effect of prepuberal medial preoptic area lesions on male rat sexual behavior.
Physiol. Behav. (1980) 24 341-346.
- LEHMAN, M.N., POWERS, J.B. & WINANS, S.S. Stria terminalis lesions alter the temporal pattern of copulatory behavior in the male golden hamster.
Behav. Brain Res. (1983) 8 109-128.
- LIEBLICH, I., SHAVIV, P. & COHEN, E. Effects of chronically elevated intake of sweet solutions on sexual behavior of male rats.
Horm. Behav. (1985) 19 278-291.
- LINCOLN, D.W. & WAKERLEY, J.B. Electrophysiological evidence for the activation of supraoptic neurones during the release of oxytocin.
J. Physiol. (Lond.) (1974) 242 533-554.
- LINCOLN, G.A. Seasonal aspects of testicular function.
In: "The Testis." BURGER, H. & DE KRETZER, D. (Eds.).
Raven Press, New York. (1981) 255-302.
- LIPP, H.P. Aggression and flight behaviour of the marmoset monkey *Callithrix jacchus*: An ethogram for brain stimulation studies.
Brain Behav. Evol. (1978) 15 241-259.
- LIPP, H.P. & HUNSPERGER, R.W. Threat, attack and flight elicited by electrical stimulation of the ventromedial hypothalamus of the marmoset monkey *Callithrix jacchus*.
Brain Behav. Evol. (1978) 15 260-293.
- LODDER, J. & ZEILMAKER, G.H. Effects of pelvic nerve and pudendal nerve transection on mating behavior in the male rat.
Physiol. Behav. (1976) 16 745-751.
- LUNN, S.F. Urinary oestrogen excretion in the common marmoset, *Callithrix jacchus jacchus*.
In: "Biology and Behaviour of Marmosets." ROTHE, H., WOLTERS, H.J. & HEARN, J.P. (Eds.).
Eigenverlag Rothe, Gottingen. (1978) 67-73.
- MACLEAN, P.D., DUA, S. & DENNISTON, R.H. Cerebral localization for scratching and seminal discharge.
Arch. Neurol. (1963) 9 485-497.

- MACLEAN, P.D. & PLOOG, D.W. Cerebral representation of penile erection. *J. Neurophysiol.* (1962) 25 29-55.
- MADLAFOUSEK, J., FREUND, K. & GROFOVA, I. Variables determining the effect of electrostimulation in the lateral preoptic area on the sexual behavior of male rats. *J. Comp. physiol. Psychol.* (1970) 72 28-44.
- MALLICK, B.N., KUMAR, V.M., CHHINA, G.S. & SINGH, B. Responses of preoptic neurons to stimulation of caudal and rostral brain stem reticular structures. *Brain Res. Bull.* (1984) 13 353-356.
- MALMNAS, C.O. Monoaminergic influence on testosterone-activated copulatory behavior in the castrated male rat. *Acta Physiol. Scand.* (1973) Suppl. 395 1-128.
- MALSBURY, C.W. Facilitation of male rat copulatory behavior by electrical stimulation of the medial preoptic area. *Physiol. Behav.* (1971) 7 797-805.
- MAS, M., RODRIGUEZ DEL CASTILLO, A., GUERRA, M., DAVIDSON, J.M. & BATTANER, E. Neurochemical correlates of male sexual behavior. *Physiol. Behav.* (1987) 41 341-345.
- MATSUKURA, S., YOSHIMI, H., SUEOKA, S., KATAOKA, K., ONO, T. & OHGUSHI, N. The regional distribution of immunoreactive β -endorphin in the monkey brain. *Brain Res.* (1978) 159 228-233.
- MATSUMOTO, A., MURAKAMI, S. & ARAI, Y. Neurotropic effects of estrogen on the neonatal preoptic area grafted into the adult rat brain. *Cell Tiss. Res.* (1988) 252 33-37.
- MATSUMURA, H., GOH, Y., VENO, R., SAKAI, T. & HAYAISHI, O. Awakening effect of PGE₂ microinjected into the preoptic area of rats. *Brain Res.* (1988) 444 265-272.
- MCCREERY, B.R. Pituitary gonadotrophin release by graded electrical stimulation of the preoptic area in the male bullfrog, *Rana catesbeiana*. *Gen. Comp. Endocrinol.* (1984) 55 367-372.
- MCINTOSH, T.K. & BARFIELD, R.J. Brain monoaminergic control of male reproductive behavior. I. Serotonin and the post-ejaculatory refractory period. *Behav. Brain Res.* (1984a) 12 255-265.
- MCINTOSH, T.K. & BARFIELD, R.J. Brain monoaminergic control of male reproductive behavior. II. Dopamine and the post-ejaculatory refractory period. *Behav. Brain Res.* (1984b) 12 267-273.

- MCINTOSH, T.K. & BARFIELD, R.J. Brain monoaminergic control of male reproductive behavior. III. Norepinephrine and the post-ejaculatory refractory period.
Behav. Brain Res. (1984c) 12 275-281.
- MCINTOSH, T.K., VALLANO, M.L. & BARFIELD, R.J. Effects of morphine, β -endorphin and naloxone on catecholamine levels and sexual behavior in the male rat.
Pharm. Biochem. & Behav. (1980) 13 435-441.
- MCNEILLY, A.S & DUCKER, H.A. Blood levels of oxytocin in the female goat during coitus and in response to stimuli associated with mating.
J. Endocrinol. (1972) 54 399-406.
- MEISEL, R.L. Effects of postweaning rearing condition on recovery of copulatory behavior from lesions of the medial preoptic area in rats.
Dev. Psychobiol. (1982) 15 331-338.
- MEISEL, R.L. Recovery of masculine copulatory behavior from lesions of the medial preoptic area: Effects of age versus hormonal state.
Behav. Neurosci. (1983) 97 785-793.
- MELIS, M.R., ARGOLAS, A. & GESSA, G.L. Oxytocin-induced penile erection and yawning: Site of action in the brain.
Brain Res. (1986) 398 259-265.
- MELIS, M.R., ARGOLAS, A. & GESSA, G.L. Apomorphine-induced penile erection and yawning: Site of action in brain.
Brain Res. (1987) 415 98-104.
- MERARI, A. & GINTON, A. Characteristics of exaggerated sexual behavior induced by electrical stimulation of the medial preoptic area in male rats.
Brain Res. (1975) 86 97-108.
- MEYER, C.C. Effects of lesions in the medial preoptic region on precocial copulation in the male chick.
Horm. Behav. (1974) 5 377-381.
- MEYERS, R. Evidence of a locus in the neural mechanisms of libido and penile potency in the septo-fornico-hypothalamic region of the human brain.
Trans. Am. neurol. Ass. (1961) 86 81-85.
- MEYERS, R. Three cases of myoclonus alleviated by bilateral ansotomy, with a note on postoperative libido and impotence.
J. Neurosurgery (1962) 19 71-81.
- MEYERSON, B.J. & TERENIUS, L. β -endorphin and male sexual behavior.
Euro. J. Pharmacol. (1977) 42 191-192.

- MICELI, M.O., FLEMING, A.S. & MALSBURY, C.W. Disruption of maternal behaviour in virgin and postparturient rats following sagittal plane knife cuts in the preoptic area-hypothalamus.
Behav. Brain Res. (1983) 9 337-360.
- MICEVYCH, P.E., SHIM PARK, S., AKESSON, T.R. & ELDE, R. Distribution of cholecystokinin-immunoreactive cell bodies in the male and female rat: I. Hypothalamus.
J. Comp. Neurol. (1987) 255 124-136.
- MICHAEL, R.P. & BONSALL, R.W. Chemical signals and primate behavior. In: "Chemical signals in vertebrates." MULLER-SCHWARZE, D. & MOZELL, M.M. (Eds.).
Plenum Press, New York (1977) 251-271.
- MICHAEL, R.P., BONSALL, R.W. & REES, H.D. Sites at which testosterone may act as an estrogen in the brain of the male primate.
Neuroendocrinol. (1987) 46 511-521.
- MICHAEL, R.P. & KEVERNE, E.B. Pheromones and the communication of sexual status in primates.
Nature (1968) 218 746-749.
- MICHAEL, R.P. & KEVERNE, E.B. Primate sex pheromones of vaginal origin.
Nature (1970) 225 84-85.
- MICHAEL, R.P., ZUMPE, D., KEVERNE, E.B. & BONSALL, R.W. Neuroendocrine factors in the control of primate behaviour.
Rec. Prog. Horm. Res. (1972) 28 665-706.
- MOLTZ, H., LEVIN, R. & LEON, M. Differential effects of progesterone on the maternal behavior of primiparous and multiparous rats.
J. Comp. physiol. Psychol. (1969) 67 36-40.
- MOLTZ, H., LUBIN, M., LEON, M. & NUMAN, M. Hormonal induction of maternal behavior in the ovariectomized nulliparous rat.
Physiol. Behav. (1970) 5 1373-1377.
- MORGANTALER, A. & CREWS, D. Role of the anterior hypothalamus-preoptic area in the regulation of reproductive behavior in the lizard, *Anolis carolinensis*: Implantation studies.
Horm. Behav. (1978) 11 61-73.
- MORTIMER, C.H., MCNEILLY, A.S. & BESSER, G.M. Gonadotrophin releasing hormone therapy.
Ann. Biol. anim. Biochem. Biophys. (1976) 16 235-243.
- MORTIMER, C.H., MCNEILLY, A.S., FISHER, R.A., MURRAY, M.A.F. & BESSER, G.M. Gonadotrophin-releasing hormone therapy in hypogonadal males with hypothalamic or pituitary dysfunction.
Br. Med. J. (1974) iv 617-621.

- MOSS, R.L. Effects of hypothalamic peptides on sex behavior in animal and man.
In: "Psychopharmacology: A generation of progress." LIPTON, M.A., DIMASCIO, A. & KILLAM, K.F. (Eds.).
Raven Press, New York. (1978) 431-440.
- MROCZEK, W.J., LEIBEL, B.A. & FINNERTY, F.A. Comparison of clonidine and methyldopa in hypertensive patients receiving a diuretic. A double-blind crossover study.
Am. J. Cardiol. (1972) 29 712-717.
- MUNRO, D.H., HORNE, H.W. & PAULL, D.P. The effect of injury to the spinal cord and cauda equina on the sexual potency of men.
New England J. Med. (1948) 239 903-911.
- MURPHY, M.R. Methadone reduces sexual performance and sexual motivation in the male Syrian golden hamster.
Pharm. Biochem. & Behav. (1981) 14 561-567.
- MURPHY, M.R. & SCHNEIDER, G.E. Olfactory bulb removal eliminates mating behavior in the male golden hamster.
Science (1970) 167 302-303.
- MYERS, B.M. & BAUM, M.J. Facilitation of copulatory performance in male rats by naloxone: Effects of hypophysectomy, 17 α -estradiol, and luteinizing hormone releasing hormone.
Pharm. Biochem. & Behav. (1980) 12 365-370.
- NADLER, R.D., WALLIS, J., ROTH-MEYER, C., COOPER, R.W. & BAULIEU, E-E. Hormones and behavior of prepubertal and peripubertal chimpanzees.
Horm. Behav. (1987) 21 118-131.
- NISHIZUKA, M. & ARAI, Y. Sexual dimorphism in synaptic organization in the amygdala and its dependence on neonatal hormone environment.
Brain Res. (1981) 212 31-38.
- NUMAN, M. Medial preoptic area and maternal behavior in the female rat.
J. Comp. physiol. Psychol. (1974) 87 746-759.
- NUMAN, M. Brain mechanisms and parental behavior.
In: "Handbook of Behavioral Neurobiology. 7. Reproduction". ADLER, N., PFAFF, D. & GOY, R.W. (Eds).
Plenum Press, New York (1985) 537-608.
- NUMAN, M. & CALLAHAN, E.C. The connections of the preoptic region and maternal behavior in the rat.
Physiol. Behav. (1980) 25 653-665.
- NUMAN, M. & NAGLE, D.S. Preoptic area and substantia nigra interact in the control of maternal behavior in the rat.
Behav. Neurosci. (1983) 97 120-139.

- NUMAN, M., ROSENBLATT, J.S. & KOMISARUK, B.R. Medial preoptic area and onset of maternal behavior in the rat.
J. Comp. physiol. Psychol. (1977) 91 146-164.
- NUMAN, M. & SMITH, H.G. Maternal behavior in rats: Evidence for the involvement of preoptic projections to the ventral tegmental area.
Behav. Neurosci. (1984) 98 712-727.
- OGAWA, S., KUDO, S., KITSUNAI, Y. & FUKUCHI, S. Increase in oxytocin secretion at ejaculation in male.
Clin. Endocrinol. (1980) 13 95-97.
- O'NEILL, T.P. & BRODY, M.J. Role for the median preoptic nucleus in centrally evoked pressor responses.
Am. J. Physiol. (1987) 252 R1165-R1172.
- ONESTI, G., BOCK, K., HEIMSOTH, V., EUN KIM, K. & MERGUET, P. Clonidine: A new antihypertensive agent.
Am. J. Cardiol. (1971) 28 74-83.
- OOMURA, Y., YOSHIMATSU, H. & AUO, S. Hypothalamic neuronal activity during sexual behavior in male monkey.
In: "Modulation of Sensorimotor Activity during Alterations in Behavioral States." BANDLER, R. (Ed.).
Alan R. Liss Inc., New York (1984) 231-250.
- ORO, A.E., SIMERLY, R.B. & SWANSON, L.W. Estrous cycle variations in levels of cholecystokinin immunoreactivity within the cells of three interconnected sexually dimorphic forebrain nuclei. Evidence for a regulatory role for estrogen.
Neuroendocrinol. (1988) 47 225-235.
- OTTINGER, M.A. & BALTHAZART, J. Brain monoamines and sexual behavior in Japanese quail: Effects of castration and steroid replacement therapy.
Behavioural Processes (1987) 14 197-216.
- PANZICA, G.C., VIGLIETTI-PANZICA, C., CALACAGNI, M., ANSELMETTI, G.C., SCHUMACHER, M. & BALTHAZART, J. Sexual differentiation and hormonal control of the sexually dimorphic preoptic nucleus in the quail.
Brain Res. (1987) 416 59-68.
- PAPEZ, J.W. A proposed mechanism of emotion.
Arch. Neurol. Psychiatry (1937) 38 725-743.
- PAXINOS, G. Interruption of septal connections: Effects on drinking, irritability, and copulation.
Physiol. Behav. (1976) 17 81-88.
- PAXINOS, G. & BINDRA, D. Hypothalamic knife cuts: Effects on eating, drinking, irritability, aggression, and copulation in the male rat.
J. Comp. physiol. Psychol. (1972) 79 219-229.

PEETERS, G., LEGROS, J.J., PIRON-BOSSUYT, C., REYNAERT, R., VANDEN DRIESSE, R. & VANNIEUWENHUYSE, E. Release of neurophysin I and oxytocin by stimulation of the genital organs in bulls. *J. Endocrinol.* (1983) 99 161-171.

PERACHIO, A., MARR, L. & ALEXANDER, M. Sexual behaviour in male rhesus monkeys elicited by electrical stimulation of preoptic and hypothalamic areas. *Brain Res.* (1979) 177 127-144.

PERRETT, D.L., SMITH, P.A.J., POTTER, D.D., MISTLIN, A.J., HEAD, A.S., MILNER, A.D. & JEEVES, M.A. Visual cells in the temporal cortex sensitive to face view and gaze direction. *Proc. R. Soc. (B)* (1985) 223 293-317.

PERRYMAN, R.L. & THORNER, M.O. The effects of hyperprolactinaemia on sexual and reproductive function in man. *J. Androl.* (1981) 2 233-242.

PFAFF, D.W. Autoradiographic localization of radioactivity in rat brains after injection of tritiated sex hormones. *Science* (1968) 161 1355-1356.

PFAFF, D.W. Estrogens and brain function. Springer-Verlag, New York. (1980).

PFAUS, J.G. & GORZALKA, B.B. Opioids and sexual behavior. *Neurosci. & Biobehav. Rev.* (1987) 11 1-34.

PHOENIX, C.H. Hypothalamic regulation of sexual behavior in male guinea pigs. *J. Comp. physiol. Psychol.* (1961) 54 72-77.

PIETRAS, R.J. & MOULTON, D.G. Hormonal influences on odor detection in rats: Changes associated with the estrous cycle, pseudopregnancy, ovariectomy, and administration of testosterone propionate. *Physiol. Behav.* (1974) 12 475-491.

PLANT, T.M., MOOR, Y., HESS, D.L., NAKAI, Y., MCCORMACK, P. & KNOBIL, E. Further studies on the effects of lesions in the rostral hypothalamus on gonadotrophin secretion in the female rhesus monkey: *Macaca mulatta*. *Endocrinol.* (1979) 105 465-473.

POHL, C.R. & KNOBIL, E. The role of the central nervous system in the control of ovarian function in higher primates. *A. Rev. Physiol.* (1982) 44 583-593.

POMERANTZ, S.M., GOY, R.W. & ROY, M.M. Expression of male-typical behavior in adult female pseudohermaphroditic rhesus: Comparisons with normal males and neonatally gonadectomized males and females. *Horm. Behav.* (1986) 20 483-500.

POWERS, J.B., NEWMAN, S.W. & BERGONDY, M.L. MPOA and BNST lesions in male Syrian hamsters: Differential effects on copulatory and chemoinvestigatory behaviors.
Behav. Brain Res. (1987) 23 181-195.

PRINS, G.S., BARTKE, A., CHANDRASHEKAR, V., REIHER, J., HODGES, S. & MEYERS, R. Effects of bromocriptine and ectopic pituitary transplants on pituitary and hypothalamic nuclear androgen receptors in the male hamster.
Neuroendocrinol. (1988) 47 236-240.

QUADAGNO, D.M., ALBELDA, S.M., MCGILL, T.E. & KAPLAN, L.J. Intracranial cycloheximide: Effect on male mouse sexual behavior and plasma testosterone.
Pharmacol. Biochem. Behav. (1976) 4 185-189.

RAISMAN, G. & FIELD, P.M. Sexual dimorphism in the preoptic area of the rat
Science (1971) 173 731-733.

RISSMAN, E.F. Gonadal influences on sexual behavior in the male musk shrew (*Suncus murinus*).
Horm. Behav. (1987) 21 132-136.

ROBERTS, W.W., STEINBERG, M.L. & MEANS, L.W. Hypothalamic mechanisms for sexual, aggressive, and other motivational behaviors in the opossum, *Didelphis virginiana*.
J. Comp. physiol. Psychol. (1967) 64 1-15.

ROBINSON, B.W. & MISHKIN, M. Penile erection evoked from forebrain structures in *Macaca mulatta*.
Arch. Neurol. (1968) 19 184-198.

ROSELLI, C.E., STADELMAN, H., HORTON, L.E. & RESKO, J.A. Regulation of androgen metabolism and luteinizing hormone-releasing hormone content in discrete hypothalamic and limbic areas of male rhesus macaques.
Endocrinol. (1987) 120 97-106.

ROSENBERG, P., HALARIS, A. & MOLTZ, H. Effects of central norepinephrine depletion on the initiation and maintenance of maternal behavior in the rat.
Pharmac. Biochem. Behav. (1977) 6 21-24.

ROSENBLATT, J.S. Nonhormonal basis of maternal behavior in the rat.
Science (1967) 136 1512-1514.

ROSENBLATT, J.S., MAYER, A.D. & SIEGEL, H.I. Maternal behavior among the nonprimate mammals.
In: "Handbook of Behavioral Neurobiology. 7. Reproduction." ALDER, N., PFAFF, D. & GOY, R.W. (Eds.).
Plenum Press, New York. (1985) 229-298.

- ROSENBLATT, J.S. & SIEGEL, H.I. Hysterectomy-induced maternal behavior during pregnancy in the rat.
J. Comp. physiol. Psychol. (1975) 89 685-700.
- RYLANDS, A.B. Infant-carrying in a wild marmoset group, *Callithrix humeralifer*: Evidence for a polyandrous mating system.
A Primatologia no. Brasil - 2. THIAGO DE MELLO, M. (Ed.).
Sociedade Brasileira de Primatologia. (1986) 131-144.
- SACHS, B.D. & MEISEL, R.L. The physiology of male sexual behaviour.
In: "The Physiology of Reproduction." KNOBIL, E. & NEILL, J. ET AL., (Eds.).
Raven Press Ltd., New York (1988) 1393-1485.
- SAR, M. & STUMPF, W.E. Autoradiographic localization of radioactivity in the rat brain after the injection of 1,2-³H-testosterone.
Endocrinol. (1973) 92 251-256.
- SCALIA, F. & WINANS, S.S. The differential projections of the olfactory bulb and accessory bulb in mammals.
J. Comp. Neurol. (1975) 161 31-56.
- SCANLON, C.E., CHALMERS, N.R. & MONTEIRO DA CRUZ, M.A.O. Changes in the size, composition and reproductive condition of wild marmoset groups (*Callithrix jacchus jacchus*) in north east Brasil.
(In press).
- SCARAMUZZI, R.T., CORKER, C.S., YOUNG, G. & BAIRD, D.T. Production of antisera to steroid hormones in sheep. Steroid immunoassay.
In: "Proceedings of the 5th Tenovus Workshop." CAMERON, E.H.D., HILLIER, S.G. & GRIFFITHS, K. (Eds).
Alpha Omega Alpha Publishing, Cardiff (1975) 111-222.
- SCHENCK, P.E. & KOOS SLOB, A. Castration, sex steroids, and heterosexual behavior in adult male laboratory-housed stump-tailed macaques (*Macaca arctoides*).
Horm. Behav. (1986) 20 336-353.
- SCHMIDT, R.S. Preoptic activation of frog mating behavior.
Behaviour (1968) 30 239-257.
- SCHMIDT, R.S. Neural correlates of frog calling: Preoptic area trigger of 'mate calling'.
J. Comp. Physiol. A. (1984) 154 847-853.
- SCHOFIELD, S.P.M. & DIXSON, A.F. Distribution of catecholamine and indoleamine neurons in the brain of the common marmoset (*Callithrix jacchus*).
J. Anat. (1982) 134 315-338.
- SCHREINER, L. & KLING, A. Rhinencephalon and behavior.
Am. J. Physiol. (1956) 184 486-490.

- SCHWARCZ, R., SCHOLZ, D. & COYLE, J.T. Structure-activity relations for the neurotoxicity of kainic acid derivatives and glutamate analogues. *Neuropharm.* (1978) 17 145-151.
- SECKL, J.R. & LIGHTMAN, S.L. Effect of naloxone on oxytocin and vasopressin release during vaginocervical stimulation in the goat. *J. Endocrinol.* (1987) 115 317-322.
- SELLERS, K.J. & BARTKE, A. Hormonal responses to female conspecifics in hyperprolactinaemic male rats and mice. *Horm. Behav.* (1987) 21 83-92.
- SHARMA, S.C., FITZPATRICK, R.J. & WARD, W.R. Coital-induced release of oxytocin in the ram. *J. Reprod. Fertil.* (1972) 31 488-489.
- SHARMA, O.P. & HAYS, R.L. Release of an oxytocic substance following genital stimulation in bulls. *J. Reprod. Fertil.* (1973) 35 359-362.
- SHARPE, R.M. & BARTLETT, J.M.S. Intratesticular distribution of testosterone in rats and the relationship to the concentrations of a peptide that stimulates testosterone secretion. *J. Reprod. Fertil.* (1985) 73 223-236.
- SHELDRIK, E.L. & FLINT, A.P.F. Circulating concentrations of oxytocin during the estrous cycle and early pregnancy in sheep. *Prostaglandins* (1981) 22 631-636.
- SHERIDAN, P.J. & WEAKER, F.J. The primate spinal cord is a target for gonadal steroids. *J. Neuropath. exp. Neurol.* (1981) 40 447-453.
- SHRABERG, D. & WEISBERG, L. Single case study: The Kluver-Bucy syndrome in man. *J. Nervous & Mental Disease* (1978) 166 130-134.
- SIEGEL, H.I. & ROSENBLATT, J.S. Hormonal basis of hysterectomy-induced maternal behavior during pregnancy in the rat. *Horm. Behav.* (1975a) 6 211-222.
- SIEGEL, H.I. & ROSENBLATT, J.S. Progesterone inhibition of estrogen-induced maternal behavior in hysterectomized-ovariectomized virgin rats. *Horm. Behav.* (1975b) 6 223-230.
- SIEGEL, H.I. & ROSENBLATT, J.S. Estrogen-induced maternal behavior in hysterectomized-ovariectomized virgin rats. *Physiol. Behav.* (1975c) 14 465-471.
- SILVERMAN, A-J. Luteinizing hormone releasing hormone containing synapses in the diagonal band and preoptic area of the guinea pig. *J. Comp. Neurol.* (1984) 227 452-458.

- SILVERMAN, A.J., ANTUNES, J.L., FERIN, M. & ZIMMERMAN, E.A. The distribution of luteinizing hormone-releasing hormone (LHRH) in the hypothalamus of the rhesus monkey. Light microscopic studies using immunoperoxidase technique. *Endocrinol.* (1977) 101 134-142.
- SIMERLY, R.B., GORSKI, R.A. & SWANSON, L.W. Neurotransmitter specificity of cells in the medial preoptic nucleus: An immunohistochemical study in the rat. *J. Comp. Neurobiol.* (1986) 246 343-363.
- SIMERLY, R.B. & SWANSON, L.W. The organization of neural inputs to the medial preoptic nucleus of the rat. *J. Comp. Neurol.* (1986) 246 312-342.
- SIRINATHSINGHI, D.J.S. Inhibitory influence of corticotropin releasing factor on components of sexual behaviour in the male rat. *Brain Res.* (1987) 407 185-190.
- SLIMP, J.C., HART, B.L. & GOY, R.W. Heterosexual, autosexual and social behavior of adult male rhesus monkeys with medial preoptic-anterior hypothalamic lesions. *Brain Res.* (1978) 142 105-122.
- SLOTNICK, B.M. Disturbances of maternal behavior in the rat following lesions of the cingulate cortex. *Behavior* (1967) 29 204-235.
- SLOTNICK, B.M. Maternal behavior deficits following forebrain lesions in the rat. *Amer. Zool.* (1969) 9 1068-1069.
- SMOTHERMAN, W.P., HENNESSY, J.W. & LEVINE, S. Medial preoptic area knife cuts in the lactating female rat: Effects on maternal behavior and pituitary-adrenal activity. *Physiol. psychol.* (1977) 5 243-246.
- SODERSTEN, P., ENEROTH, P., HANSSON, T., MODE, A., JOHANSSON, D., NASLUND, B., LIANG, T. & GUSTAFSSON, J-A. Activation of sexual behaviour in castrated rats: The role of oestradiol. *J. Endocrinol.* (1986) 111 455-462.
- SOFRONIEW, M.V. Projections from vasopressin, oxytocin, and neurophysin neurons to neural targets in the rat and human. *J. Histochem. & Cytochem.* (1980) 28 475-478.
- SOULAIRAC, A. & SOULAIRAC, M.L. Effects des lesions hypothalamiques sur le comportement sexuel et le tractus genital du rat male. *Annales d'Endocrinologie* (1956) 17 731-745.

STEELE, M.K., ROWLAND, D. & MOLTZ, H. Initiation of maternal behavior in the rat: Possible involvement of limbic norepinephrine. *Pharmac. Biochem. Behav.* (1979) 11 123-130.

STEPHAN, H., BARON, G. & SCHWERDTFEGGER, W.K. The brain of the common marmoset. A stereotaxic atlas. Springer-Verlag, Berlin (1980)

STEVENSON, M.F. & POOLE, T.B. An ethogram of the common marmoset (*Callithrix jacchus jacchus*): General behavioural repertoire. *Anim. Behav.* (1976) 24 428-451.

STONEHAM, M.D., EVERITT, B.J., HANSEN, S., LIGHTMAN, S.L. & TODD, K. Oxytocin and sexual behaviour in the male rat and rabbit. *J. Endocrinol.* (1985) 107 97-106.

SUTCLIFFE, A.G. & POOLE, T.B. Scent marking and associated behaviour in captive common marmosets (*Callithrix jacchus jacchus*) with a description of the histology of scent glands. *J. Zool. Lond.* (1978) 185 41-56.

SVARE, B., BARTKE, A., DOHERTY, P., MASON, I., MICHAEL S.D. & SMITH, M.S. Hyperprolactinaemia suppresses copulatory behavior in male rats and mice. *Biol. Reprod.* (1979) 21 529-535.

SVENSSON, L. & HANSEN, S. Spinal monoaminergic modulation of masculine copulatory behavior in the rat. *Brain Res.* (1984) 302 315-321.

SWAAB, D.F. & FLIERS, E. A sexually dimorphic nucleus in the human brain. *Science* (1985) 228 1112-1115.

SWANSON, L.W., MOGENSEN, G.J., SIMERLY, R.B. & WU, M. Anatomical and electrophysiological evidence for a projection from the medial preoptic area to the 'mesencephalic and subthalamic locomotor regions' in the rat. *Brain Res.* (1987) 405 108-122.

SZECHTMAN, H. CAGGIULA, A.R. & WULKAN, D. Preoptic knife cuts and sexual behavior in male rats. *Brain Res.* (1978) 150 569-591.

TAN, G.J.S. & KWAN, T.K. Effect of oxytocin on plasma testosterone levels in the male macaque (*Macaca fascicularis*). *Contraception* (1987) 36 359-367.

TARDIF, S.D., CARSON, R.L. & GANGAWARE, B.L. Comparison of infant care in family groups of the common marmoset (*Callithrix jacchus*) and the cotton-top tamarin (*Saguinus oedipus*). *Am. J. Primatol.* (1986) 11 103-110.

TERBORGH, J. & GOLDIZEN, A.W. On the mating system of the cooperatively breeding saddle-backed tamarins (*Saguinus fuscicollis*). *Behav. Ecol. Sociobiol.* (1985) 16 293-299.

TERKEL, J., BRIDGES, R.S. & SAWYER, C.H. Effects of transecting lateral neural connections of the medial preoptic area on maternal behavior in the rat: Nest building, pup retrieval and prolactin secretion. *Brain Res.* (1979) 169 369-380.

TERKEL, J. & ROSENBLATT, J.S. Humoral factors underlying maternal behavior at parturition: Cross transfusion between freely moving rats. *J. Comp. physiol. Psychol.* (1972) 80 365-371.

TERLECKI, L.J. & SAINSBURY, R.S. Effects of fimbria lesions on maternal behavior in the rat. *Physiol. Behav.* (1978) 21 89-97.

TERZIAN, H. & DALLE ORE, G. Syndrome of Kluver and Bucy reproduced in man by bilateral removal of the temporal lobes. *Neurology* (1955) 5 373-380.

THIESSEN, D.D., YAHR, P.I. & OWEN, K. Regulatory mechanisms of territorial marking in the Mongolian gerbil. *J. Comp. physiol. Psychol.* (1973) 82 382-393.

TOBET, S.A. & BAUM, M.J. Role for prenatal estrogen in the development of masculine sexual behavior in the male ferret. *Horm. Behav.* (1987) 21 419-429.

TODD, K. & LIGHTMAN, S.L. Oxytocin release during coitus in male and female rabbits: Effect of opiate receptor blockade with naloxone. *Psychoneuroendocrinol.* (1986) 11 367-371.

TONJES, R., DOCKE, F. & DORNER, G. Effects of neonatal intracerebral implantation of sex steroids on sexual behaviour, social play behaviour and gonadotropin secretion. *Exp. Clin. Endocrinol.* (1987) 90 257-263.

TORRAN-ALLERAND, C.D. Sex steroids and the development of the newborn mouse hypothalamus and preoptic area *in vitro* : Implications for sexual differentiation. *Brain Res.* (1976) 106 407-412.

TOWNSEND, D.S. & MOGER, W.H. Plasma androgen levels during male parental care in a tropical frog (*Eleutherodactylus*). *Horm. Behav.* (1987) 21 93-99.

TWIGGS, D.G., POPOLOW, H.B. & GERALL, A.A. Medial preoptic lesions and male sexual behavior: Age and environmental interactions. *Science* (1978) 200 1414-1415.

- VAN DIS, H. & LARSSON, K. Induction of sexual arousal in the castrated male rat by intracranial stimulation.
Physiol. Behav. (1971) 6 85-86.
- VAUGHAN, E. & FISHER, A.E. Male sexual behavior induced by intracranial electrical stimulation.
Science (1962) 137 758-760.
- VOGT, J.L., CARLSON, H. & MENZEL, E. Social behavior of a marmoset (*Saguinus fuscicollis*) group. I. Parental care and infant development.
Primates (1978) 19 715-726.
- WADA, M. & GORBMAN, A. Mate calling induced by electrical stimulation in freely moving Leopard frogs, *Rana pipiens*.
Horm. Behav. (1977) 9 141-149.
- WAYNER, M.J., BARONE, F.C., SCHAROUN, S.L., GUEUARA-AGUILAR, R. & AGUILAR-BATURONI, H.U. Limbic connections to the lateral preoptic area: A horseradish peroxidase study in the rat.
Neurosci. Biobehav. Rev. (1983) 7 375-384.
- WEBER, R.F.A., OOMS, M.P. & VREEBURG, J.T.M. Effects of a prolactin-secreting tumour on copulatory behavior in male rats.
J. Endocrinol. (1982) 93 223-229.
- WHEELER, J.M. & CREWS, D. The role of the anterior hypothalamus-preoptic area in the regulation of male reproductive behavior in the lizard, *Anolis carolinensis*: Lesion studies.
Horm. Behav. (1978) 11 42-60.
- WINANS, S.S. & POWERS, J.B. Neonatal and two-stage olfactory bulbectomy: Effects on male hamster sexual behavior.
Behav. Biol. (1974) 10 461-471.
- YAHN, P., COMMINS, D., JACKSON, J.C. & NEWMAN, A. Independent control of sexual and scent marking behaviors of male gerbils by cells in or near the medial preoptic area.
Horm. Behav. (1982) 16 304-322.
- YAHN, P. & ULIBARRI, C. Polyadenylated and nonadenylated messenger RNA and androgen control of sexual behavior and scent marking in male gerbils.
Horm. Behav. (1987) 21 53-64.
- YOSHIMATSU, H. Hypothalamic regulatory mechanism of sexual behaviour in the male monkey.
Fukuoka Acta Medica (1983) 74 89-100.
- ZOELLER, R.T. & MOORE, F.L. Brain arginine vasotocin concentrations related to sexual behaviors and hydromineral balance in an amphibian.
Horm. Behav. (1988) 22 66-75.

ZUMPE, D. & MICHAEL R.P. Effects of testosterone on the behavior of male cynomolgus monkeys (*Macaca fascicularis*).
Horm. Behav. (1985) 19 265-277.

PUBLICATIONS

PAPERS

DIXSON, A.F. & LLOYD, S.A.C. The hormonal and hypothalamic control of primate sexual behaviour.
Symp. Zool. Soc. Lond. (1988) 60 81-117.

LLOYD, S.A.C. & DIXSON, A.F. Effects of hypothalamic lesions upon the sexual and social behaviour of the male common marmoset (*Callithrix jacchus*).
Brain Res. (1988) 463 317-329.

DIXSON, A.F. & LLOYD, S.A.C. Effects of male partners upon proceptivity in ovariectomised estradiol-treated marmosets (*Callithrix jacchus*).
Horm. Behav. (1989) (in press).

LLOYD, S.A.C. & DIXSON, A.F. Effects of hypothalamic lesions upon sexual and aggressive behaviour in the common marmoset (*Callithrix jacchus*).
Primate Report (1989) (in press).

PRESENTATIONS

MILES, S.A.C. & DIXSON, A.F. Anteromedial hypothalamic lesions, sexual behaviour and plasma testosterone levels in male marmosets (*Callithrix jacchus*).
SSF & BNG Joint Winter Meeting, December 1986.

MILES, S.A.C. & DIXSON, A.F. Effects of anteromedial hypothalamic lesions upon sexual and social behaviour in male marmosets (*Callithrix jacchus*).
SCAB Meeting, April 1987.

LLOYD, S.A.C. The preoptic area controls parental behaviour in a primate.
SCAB & ASAB Joint Easter Meeting, April 1988.

LLOYD, S.A.C. & DIXSON, A.F. Effects of hypothalamic lesions upon sexual and aggressive behaviour in the male common marmoset (*Callithrix jacchus*).
IPS Congress, July 1988.

LLOYD, S.A.C. & DIXSON, A.F. Preoptic area (POA) lesions affect parental and sexual behaviour in male marmosets (*Callithrix jacchus*).
IPS Congress, July 1988.